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The Journal of Laboratory and Clinical Medicine

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ORIGINAL ARTICLES

EXPERIMENTAL CLOUDY SWELLING OF THE KIDNEY IN THE RABBIT.*

BY W. RAY SHANNON, MINNEAPOLIS, MINN.

VIRCHOW[1] introduced the term "cloudy swelling" ("trübe Schwellung") in 1858 to describe a condition often found in the parenchymatous organs, such as the liver and kidney, in which the organ is swollen and light colored. It frequently presents a cooked appearance. When examined under the microscope in the fresh state, the cells are seen to be opaque, due to the presence of small albuminous granules. These granules may almost entirely hide the nucleus. The gross picture is due to the presence of these albuminous granules within the cells.

This classical description has been adopted with minor modifications by the majority of observers since Virchow's time. It has been copied into practically all of the modern text books of pathology.

Many pathologists often make a diagnosis of cloudy swelling upon the gross examination alone. But, if such diagnoses are checked by a microscopic study, it will frequently be found that there is no increase of albuminous granules. Turbidity and swelling of an organ are not always associated with increase of albuminous granules. On the other hand there may be a marked increase of albuminous granules in the cells without the presence of turbidity or swelling of the organ. It is, therefore, evident that cloudy swelling as commonly described includes more than one type of pathologic process. No important advance can be made until the several phenomena have been studied separately. With this in mind, I have undertaken the study of experimental cloudy swelling of the kidney, paying particular attention to the albuminous granules.

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*I wish to thank Dr. E. T. Bell, at whose suggestion and under whose supervision this work was carried out, for his helpful suggestions and enthusiastic interest. I am indebted to Mr. A. Lundquist for assistance in technic.

Cohnheim[2] adds to Virchow's description that the condition is more easily recognized grossly than microscopically in organs such as the liver and kidney, in which the cells normally contain some granules. He states that cloudy swelling occurs also in muscle (heart muscle, especially) and in the mammary glands.

Klebs[3] and Ribbert[4] agree with Cohnheim that cloudy swelling is more readily recognized grossly than microscopically.

Rindfleisch,[5] Birch-Hirschfeld,[6] Thoma,[7] Ziegler,[8] Albrecht,[9] Landsteiner,[10] Adami,[11] and von Gierke[12] all adhere closely to Virchow's description.

Benario[13] stated that fatty granules were always to be found together with the albuminous granules in cloudy swelling.

Volhard and Fahr,[14] working on the human kidney, find that the gross picture cannot be relied upon. Swelling is sometimes present and sometimes not, and the degree of cloudiness is very variable. The microscopic picture is more dependable. The epithelium of the convoluted tubules is swollen. The lumina of these tubules are decreased in size if not entirely obliterated by the swollen cells. Albuminous material is to be found in precipitated stringy masses in the lumina of some of the tubules and Bowman's capsules. The Altmann granules are enlarged and very irregular in arrangement. The rods are present in some cells.

Bell[15] noted that organs will sometimes show this gross appearance of cloudy swelling when there is no increase of albuminous granules; and, conversely, that sometimes, especially in the kidney, there may be a marked increase of albuminous granules in the absence of any gross indication. He recommends that the term "cloudy swelling" be used for gross description only, and that a modifying phrase be added to indicate the microscopic picture accompanying it.

A number of observers have studied experimental cloudy swelling in animals. Favre[16] in 1892 produced the condition in rabbit kidneys by removing the opposite kidney or by tying off its vein or ureter. He described an intense clouding of some of the convoluted tubules of the functionating kidney, due to the accumulation of albuminous granules within the cells.

Schilling[17] produced cloudy swelling in rabbit kidneys by tying the opposite renal vein for 48 hours. At the end of this time the unoperated kidney showed a swollen grayish-yellow cortex which was easily distinguishable from the blood-red medulla. Crushed preparations showed, microscopically, opaque tubules which were filled with albuminous granules. These granules hid the nuclei almost completely. He found no fat present.

Albrecht[9] also used rabbits for the study of experimental cloudy swelling of the kidney. He produced the condition by the following procedure. The renal artery was clamped off for two hours, after which time the clamp was removed. Within two or three hours more the clamp was again replaced and left for twenty-four hours. He obtained some fatty granules as well as albuminous granules in the tubules.

Bell[15] found that there was considerable discrepancy between the gross and microscopic appearances of experimental as well as human cloudy swelling.

The term "cloudy swelling" will be used in this paper to designate a turbid swollen organ without regard to the microscopic picture that may be present.

MATERIAL AND METHODS.

Since the number and character of the granules in the renal tubules of different animals vary considerably, it was decided to use but one species. Rabbits were used exclusively. All kidney operations were made through lumbar incisions. The left kidney was operated on most frequently because it is more accessible than the right.

Compensatory changes in the kidney were studied by removing the opposite kidney or by ligature of its ureter. One form of cloudy swelling was produced by subcutaneous injections of tartaric acid. Cloudy swelling was also produced by placing sterile macerated liver in the peritoneal cavity. It was found that large subcutaneous abscesses will produce a marked increase in albuminous granules in the kidney in the course of one or two weeks. Rabbits were therefore given pasteurella and proteus infections to produce abscesses. They were killed at various stages of the infectious process. These several procedures will be explained in more detail under the separate headings.

Thin pieces of fresh kidney were fixed in Zenker-formol and 10 per cent formalin. Both these reagents preserve the granules well, but Zenker-formol gives a better cytoplasmic fixation than the formalin alone. Neither Zenker's nor Bouin's solutions could be used because the acetic acid in them destroys the granules rapidly.

Zenker-formol material was found most useful for histological study. Paraffin sections were cut at three micra and stained with iron-hematoxylin and other stains. Frozen sections were prepared from the formalin-fixed material and stained for fat. In every case crushed preparations of the fresh kidney were examined to control the fixed material and to study the effect of various reagents upon the albuminous granules.

THE NORMAL KIDNEY.

Crushed preparations of the fresh cortex usually show, microscopically, a few dark tubules and a large number of light tubules. Upon high magnification the dark tubules are seen to be filled with rather large weakly refractive granules or droplets. Upon the addition of 5 per cent NaOH or KOH these granules can be seen to disappear suddenly, like the bursting of a bubble, as the reagent comes into contact with them. This leaves the tubules light and the nuclei more clearly visible. These granules are not entirely confined to the dark tubules, for a few scattered ones are noticeable in the light tubules as well. The rods of Heidenhain can also be made out in the fresh preparations in some cases. One occasionally finds a kidney which shows no distinctly dark tubules but high magnification will always reveal occasional granules within the cells.

The granules cannot be demonstrated in tissues which have been fixed with Zenker's or Bouin's fixing fluids. Apparently the acetic acid in these reagents destroys them. Ten per cent formalin or Zenker-formol preserves the granules very satisfactorily, however.

In tissues fixed with Zenker-formol or 10 per cent formalin and stained with iron-hematoxylin, the granules are well shown (Fig. 1). They appear as darkly staining spherical bodies. They are often arranged in rows between the rods. Sometimes, however, they are found in the inner clear portion of the cells. The dark tubules are very conspicuous in stained preparations (Fig. 2).

The rods of Heidenhain can be seen in the basal portion of the cells. They are apparently independent of the granules under consideration. The inner portion of the cells is clear and has a convex inner border projecting into the lumen. The lumina of the tubules are generally narrow and stellate-shaped, due to the rounded inner borders of the cells. In some of the tubules there

Fig. 1.

Fig. 2.

Fig. 1.—Normal kidney. Zenker-formol. Iron-hematoxylin. A dark tubule is shown with parts of adjacent light tubules. X 660.

Fig. 2.—Normal kidney. Zenker-formol. Iron-hematoxylin. Showing the relative number of dark tubules. X 100.

may be no visible lumen, the inner borders of the cells meeting and entirely obliterating it.

Albrecht[9] described the granules under consideration in the renal epithelium of rabbits and stated that he could find no previous mention of them in the literature. He thought that they were of some functional significance. Disse,[18] however, in 1892 described granules in the cortical epithelium of the rabbit kidney which correspond closely to these. He did not examine them in the fresh tissue, nor did he attribute any functional importance to the granules.

Rothstein[19] described these granules in the kidneys of mammals in 1890. In the fresh crushed preparations they appeared as definite refractive granules with darkly limited periphery, sometimes isolated and sometimes in groups within the cells. In fixed and stained preparations they were sometimes seen to be spherical, sometimes oval, and even sometimes spindle-shaped. He evidently considered them of functional importance.

Modragowski[20] mentions these granules in normal rabbit kidneys and gives them some functional significance.

Hirsch[21] gives an accurate description of the granules and dark tubules of the normal kidney. The number of granules depends upon the functional condition of the tubules.

Bell[15] also described these granules in the renal epithelium of the normal rabbit. He studied them in fresh preparations only.

No one has proven definitely that these granules have any functional significance; but they are invariably present in the normal kidney.

COMPENSATORY CHANGES IN THE KIDNEY.

Favre, Schilling, and Albrecht have all recorded the production of compensatory cloudy swelling in rabbit kidneys by the removal of the opposite kidney or by tying its vein or ureter. Favre did not describe the gross changes. He says that he obtained a clouding in some of the tubules, apparently not knowing that this is present in the normal. Schilling states that grossly the cortex was of a grayish-yellow color, easily distinguishable from the blood-red medulla. Microscopically, the tubules were opaque, due to the accumulation of albuminous granules within the cells. This author also apparently overlooked the fact that some of the tubules are dark in the normal kidney. Albrecht does not give a description of his findings.

Hirsch described an increase in the albuminous granules one week after the removal of the opposite kidney, but did not consider the gross changes worth mentioning.

Bell was unable to produce a definite gross cloudy swelling by removing the opposite kidney. From microscopic examination of fresh tissues he was not certain that there was an increase in the number of dark tubules over the normal.

EXPERIMENTS.

Experiment 1.—Rabbit; male; left kidney removed. Twenty-four hours later the animal was killed and the right kidney examined.

Gross examination. The left kidney is normal in appearance. The right kidney is swollen and congested, but no cloudiness can be seen.

Microscopic examination. No certain differences can be made out in the fresh specimens. The fixed and stained material shows a few more dark tubules in the right than in the left kidney.

Experiment 2.—Rabbit; male, weight 1,080 grams; left kidney removed. Forty-eight hours later the animal was killed and the right kidney examined.

Gross examination. Left kidney normal. The right kidney is somewhat swollen and the cut surface is moist, but there is no distinct cloudiness present.

Microscopic examination. The right kidney shows definitely more dark tubules than the left. There is also an increase in albuminous granules throughout the lighter tubules in the right kidney.

Experiment 3.—Rabbit; male; weight 2,910 grams; removed left kidney. Forty-eight hours later the animal was killed.

Gross examination. Left kidney normal. The right kidney is swollen and its cut surface is slightly pale.

Microscopic examination. The right kidney contains at least two or three times as many dark tubules as the left. The granules in the right kidney are larger than those in the left.

Experiment 4.—Rabbit; female; weight 2,000 grams. The right ureter was ligatured. One week later the animal was killed and the left kidney examined.

Gross examination. Possibly swollen. No cloudiness was detectable.

Microscopic examination. The fresh tissue shows no certain increase in the number of dark tubules. Fixed and stained material shows no increase over the normal number of dark tubules.

These experiments show that a moderate increase in the number of granules occurs, temporarily at least, after the removal of the opposite kidney. This is so slight as to be indistinguishable with certainty in the fresh specimens. It is only after a comparison of fixed and stained preparations that it can be determined. The gross changes are so slight that they cannot be recognized with certainty.

CLOUDY SWELLING PRODUCED BY TARTRATES.

Rabbits were given subcutaneous injections of racemic tartaric acid neutralized with sodium carbonate, in varying doses from .05 gm. per kilo to .15 gm. per kilo. At different times after the injection the rabbits were killed and their kidneys examined. The earlier stages were studied especially, since the later stages are of a frankly degenerative character and since many authors hold that parenchymatous nephritis passes through a preliminary stage of cloudy swelling.

Experiment 5.—Rabbit; male; weight 1,500 grams. Injected subcutaneously with 0.1 gm. racemic tartaric acid. The right kidney was removed one hour later. Two hours after the injection the animal was killed.

Right kidney: gross examination. Kidney appears normal.

Microscopic examination. Dark tubules are present in the fresh preparation. Paraffin sections show a few hydropic cells. Otherwise the appearances are normal.

Left kidney: gross examination. Cut surface is slightly pale.

Microscopic examination. Dark tubules are seen in the fresh preparations. Paraffin sections show nothing abnormal except a few hydropic cells.

Experiment 6.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with 0.1 gm. racemic tartaric acid. Killed three hours later.

Gross examination. No definite changes.

Microscopic examination. Crushed preparations of fresh tissue show no dark tubules. In paraffin sections stained with iron-hematoxylin most of the convoluted tubules show a few granules. There are no dark tubules. There are a few hydropic tubules in the peripheral part of the cortex.

Experiment 7.—Rabbit; male; weight 1,800 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. The left kidney was removed two hours later. Twenty-four hours after the injection the animal was killed.

Left kidney: gross examination. The kidney is swollen. The cut surface is succulent and a little pale.

Microscopic examination. The fresh crushed preparations show a few slightly dark tubules. These are not so prominent as in the normal. Paraffin sections show some dark tubules. The lighter tubules also contain a few granules. Hydropic tubules are noted especially in the peripheral part of the cortex.

Experiment 8.—Rabbit; female; weight 1,570 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. The right kidney was removed three hours later. Twenty-four hours after the injection the animal was killed.

Right kidney: gross examination. The organ is slightly swollen. The cut surface is slightly paler than normal.

Microscopic examination. In the fresh preparations most of the tubules show a few granules. There are no distinctly dark tubules to be seen. Paraffin sections show extreme hydropic degeneration of the convoluted tubules. There are a few tubules which do not show this change, but none of the dark tubules are present. The normal tubules show a few scattered granules.

Left kidney: gross examination. The organ is swollen and the cut surface is distinctly cloudy.

Microscopic examination. The fresh specimen shows no dark tubules. Paraffin sections show some dilated tubules at the periphery of the cortex. These contain casts and cellular debris. There are some necrotic tubules at the periphery of the cortex. No dark tubules are present. There are no hydropic cells.

Experiment 9.—Rabbit; male; weight 1,330 grams. Injected subcutaneously with 0.2 gm. racemic tartaric acid. Killed twenty-four hours later.

Gross examination. Kidneys are swollen. The cut surfaces are succulent and a little paler than normal.

Microscopic examination. The fresh preparation shows a few dark tubules. These contain some fatty granules beside a few albuminous granules. Paraffin sections show some dilated tubules which contain casts and cellular debris. There are a number of dark tubules present. The rods are prominent in most of the tubules.

Experiment 10.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with 0.3 gm. racemic tartaric acid. The right kidney was removed twenty-four hours later. Forty-eight hours after the injection the animal was killed.

Right kidney: gross examination. The organ is swollen and the cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh specimen shows a large number of tubules filled with fat droplets. Paraffin sections show a large number of dilated tubules containing casts and cellular debris. There are a few of the dark tubules present. There are some necrotic tubules at the periphery of the cortex. A few convoluted tubules contain large deeply staining granules, apparently different from those in the dark tubules. Fat stain shows a large amount of fat in both the collecting and the convoluted tubules.

Left kidney: gross examination. The organ is swollen. The cut surface is only slightly pale.

Microscopic examination. There are a few fatty tubules present. Paraffin preparations show fewer dilated tubules than the right. There are some of the dark tubules present.

Experiment 11.—Rabbit; male; weight 1,970 grams. Injected subcutaneously with .3 gm. racemic tartaric acid. The left kidney was removed twenty-four hours later. Forty-eight hours after the injection the animal was killed.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh preparation shows a large amount of fat throughout the collecting and convoluted tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is slightly pale.

Microscopic examination. The fresh preparation shows a few fatty tubules. Paraffin sections show only a few dilated tubules. There are some dark tubules present. Most of the convoluted tubules contain a small amount of fat.

Experiment 12.—Rabbit; male; weight 2,050 grams. Injected subcutaneously with .3 gm. racemic tartaric acid. The left kidney was removed 7 hours later. Forty-eight hours after the injection the right kidney was removed.

Left kidney: gross examination. The organ is possibly swollen. The cut surface is succulent and pale.

Microscopic examination. The fresh preparation shows a few dark tubules. Some of the tubules contain fatty granules. Paraffin preparations show a large number of hydropic tubules. There are some dark tubules. There are dilated tubules containing casts and cellular debris.

Right kidney: gross examination. The organ is swollen. The cut surface is moist and slightly pale.

Microscopic examination. There are a few dark tubules in the fresh preparation.

Paraffin sections show no hydropic tubules. The larger number of tubules appear normal and the dark tubules are present. There are a few extremely dilated tubules which contain casts and cellular debris.

From the above experiments it will be seen that the minimum effective dose produces no gross changes in the kidneys. Larger doses, however, give a definite cloudy swelling at the end of twenty-four hours. The organ cannot be said to have a "cooked appearance" but it nevertheless is swollen and turbid. The cloudiness and swelling are in no way due to the presence of albuminous granules. The albuminous granules are never increased in number above the normal and even, at times, seem to be decreased. The opacity of the kidneys is apparently due, for the most part, to anemia, fatty droplets, and necrosis and disintegration of the cells.

CLOUDY SWELLING PRODUCED BY THE INTRODUCTION OF AUTOLYZED LIVER INTO THE PERITONEAL CAVITY.

Severe renal injury may be produced by introducing a large quantity of autolyzed liver into the body.

A rabbit's liver was removed sterilely and cut up into fine pieces. Sterile physiological saline was added to this and the tissue was allowed to autolyze in the incubator for several hours. This fluid was injected into the peritoneal cavity.

Experiment 13.—Rabbit; female; weight 1,665 grams. The urine was normal. Twenty c.c. of fluid obtained by macerating a sterile rabbit liver over night with 50 c.c. of sterile physiological saline at 37° C. was injected intraperitoneally. Four hours later the urine showed numerous hyaline casts and considerable albumin. The left kidney was removed. The animal died two days later. The urine remained about the same.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately cloudy.

Microscopic examination. The fresh preparation shows no distinctly dark tubules. Some of the tubules contain a few albuminous granules, however. Paraffin preparations show a few dilated convoluted tubules. These contain sloughed off portions of cells within their lumina. The greater number of tubules appear normal. No dark tubules are present but some tubules contain a few darkly staining granules.

Right kidney: gross examination. The organ is darkened by postmortem change.

Microscopic examination. The fresh specimen shows no dark tubules although some of the tubules contain a few albuminous granules. Paraffin preparations show a large number of the tubules containing albuminous material within their lumina. There are no dark tubules but some contain a few granules.

Experiment 14.—Rabbit; male; weight 1,600 grams. Examination of the urine showed no albumin. Seventy-four gm. of sterile rabbit liver which had been macerated for five hours with 25 c.c. of sterile physiological saline was placed in the peritoneal cavity. Eighteen hours later the left kidney was removed. The urine was cloudy with granular casts and contained a large amount of albumin. Eight days later the animal died. During this time the urine remained about the same.

Left kidney: gross examination. The organ is swollen. The cut surface is extremely cloudy and succulent.

Microscopic examination. The fresh preparation shows no dark tubules. Paraffin sections show a pronounced parenchymatous nephritis but no dark tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is succulent and moderately pale.

Microscopic examination. The fresh specimen shows no dark tubules. There are a large number of fatty granules present. Paraffin sections show considerable interstitial edema. There are a few tubules which contain some albuminous granules. Some of the tubules are dilated and contain casts and cellular debris.

No increase in the number of albuminous granules occurred in these experiments. Instead, the granules entirely disappeared and a very pronounced parenchymatous nephritis resulted. In spite of the disappearance of the granules an extreme degree of cloudy swelling occurred. This must be taken as further proof that the swelling and clouding of the kidney and the accumulation of albuminous granules within the cells may be the results of distinctly different pathological processes.

(Whether the changes in the kidney were due to autolyzed proteins or to the bile salts present was not determined.)

CLOUDY SWELLING PRODUCED BY INFECTIONS.

In this group of experiments the conditions simulate some of the human diseases in which a cloudy swelling may occur. Bell mentioned the occurrence of an albuminous degeneration (cloudy swelling) in rabbit kidneys in a case of infection.

Cultures taken from a large pasteurella abscess were used to inoculate other rabbits. The animals were weighed each day and the progress of the infection was measured by the loss in weight. By killing the animals at different stages of the infectious process different degrees of cloudy swelling were obtained.

Most of the cases studied were pasteurella infections, since these organisms infect rabbits very readily. Some animals with proteus abscesses and one with a streptococcus infection were studied. The renal changes were similar in all three types of infections.

Experiment 15.—Rabbit; male; weight 1,040 grams. Inoculated subcutaneously with pasteurella. An abscess developed. Thirteen days later the animal was killed. Weight 890 grams.

Kidneys: gross examination. There are no marked changes from the normal.

Microscopic examination. The fresh specimen shows a large number of dark tubules. There are no fatty granules. Paraffin sections show considerably less than half the tubules dark (Fig. 3). These are crowded with granules which tend, in some tubules, to be arranged in rows in the basal part. The rods are present between the granules and are very prominent in the clear tubules. The cells of the tubules are swollen so that no lumina are visible. Frozen sections stained with sudan iii show that no fat is present.

Experiment 16.—Rabbit; male; weight 1,900 grams. The animal was inoculated subcutaneously with pasteurella. A large abscess developed. Fourteen days later the animal died. Weight 1,300 grams.

Kidneys: gross examination. The organs show some postmortem discoloration. They are not swollen.

Microscopic examination. The fresh specimen shows numerous dark tubules filled with albuminous granules. No fatty granules are present. Paraffin sections show numerous dark tubules filled with deeply staining granules. These are confined to the basal part of the cells in only a few tubules. The rods are not present. No fat can be demonstrated in frozen sections.

Experiment 17.—Rabbit; male; weight 2,010 grams. Phenolsulphonephthalein test, 75 per cent for the first two hours. The urine was normal. Inoculated subcutaneously with pasteurella. An abscess developed. Ten days later phenolsulphonephthalein test was 75 per cent. Two days later an examination of the urine showed a faint trace of albumin and a few hyaline casts. Thirteen days after inoculation the animal died. Weight 1,360 grams.

Kidneys: gross examination. The organs show some postmortem discoloration. They are not swollen.

Microscopic examination. The fresh specimen shows numerous dark tubules contain-

ing albuminous granules. Paraffin sections show the cells of slightly less than half the tubules filled with albuminous granules. A few of the tubules have wide lumina and fragmented cells. There is often albuminous material within the lumina of the tubules. The rods are not present.

Experiment 18.—Rabbit; male; weight 1,470 grams. The rabbit had had a spontaneous abscess on the jaw which had been noticed for about a month. Nine days after I received the rabbit it was killed. Weight 1,170 grams.

Kidneys: gross examination. The organs were not swollen. The cut surfaces are moderately cloudy.

Microscopic examination. The fresh preparation shows a large number of dark tubules which are filled with albuminous granules. Paraffin preparations show the cells of slightly more than half the tubules filled with deeply stained granules. The rods of Heidenhain are prominent in places between the granules. No fat is present.

Fig. 3.—Exp. 15. Kidney from a case of chronic infection. Zenker-formol. Iron-hematoxylin. There is a considerable increase in the number of dark tubules. Photomicrograph. X 80.

Experiment 19.—Rabbit; male; weight 2,000 grams. The left kidney was removed. One week later the rabbit was inoculated with *B. proteus*. A diffuse infection resulted. Eight days after the inoculation the animal died.

Right kidney: gross examination. The organ is markedly swollen. The cut surface is succulent and slightly pale.

Microscopic examination. The fresh preparation shows numerous dark tubules filled with albuminous granules. Some of these granules are very large. Paraffin sections show the cells of slightly more than one-half of the tubules filled with deeply staining granules. The rods are not present. (The rabbit had been dead for some time when it was found.) The lumina of most of the tubules are obliterated by the swollen cells. No fat can be demonstrated.

Experiment 20.—Rabbit; female; weight 1,850 grams. Inoculated subcutaneously with pasteurella. An extremely large abscess developed. Nine days later the left kidney was removed. Weight 1,330 grams. The next day the animal was killed.

Left kidney: gross examination. The organ is swollen. There is no noticeable cloudiness.

Microscopic examination. The fresh specimen shows numerous dark tubules which are filled with albuminous granules. These granules are very irregular in size. Paraffin preparations show a little less than one-half the tubules dark. These are crowded with darkly staining granules. The granules are very large in certain tubules. The rods of Heidenhain are present between the granules in the basal portion of the cells. Sections stained for fat show a little present in a few of the convoluted and collecting tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is slightly cloudy.

Microscopic examination. The microscopic examination shows a few more dark tubules than in the left kidney. Otherwise they are the same.

Experiment 21.—Rabbit; male; weight 1,520 grams. Phenolsulphonephthalein test, 80 per cent. Inoculated subcutaneously with *B. proteus*. Six days later, phthalein test, 75 per cent. Nine days after inoculation, phthalein test, 50 per cent. Killed ninth day after inoculation. Weight, 1,200 grams.

Kidney: gross examination. The organ is swollen. The cut surface is succulent and cloudy.

Microscopic examination. Fresh preparations show a large number of dark tubules present which contain albuminous granules. Paraffin preparations show over one-half of the tubules filled with darkly staining granules. The rods are well preserved. Fat stains show only a very few fatty granules in some of the tubules.

Experiment 22.—Rabbit; male; weight 1,610 grams. Phenolsulphonephthalein test, 70 per cent. The rabbit was inoculated subcutaneously with *B. proteus*. A diffuse infection resulted. Three days later the phthalein test was the same. Five days after the inoculation the animal died. Weight 1,090 grams.

Kidneys: gross examination. No swelling is present. The cut surfaces are succulent but not cloudy.

Microscopic examination. The fresh preparation shows a large number of moderately dark tubules. Paraffin preparations show that about half of the tubules are dark. The number of granules is less than in pronounced cases. Considerable fat was demonstrated in some of the convoluted tubules and in the collecting tubules.

Experiment 23.—Rabbit; male; weight 1,910 grams. Urine examination showed nothing abnormal. Phenolsulphonephthalein test, 80 per cent. Inoculated subcutaneously with *B. proteus*. A large abscess developed. Nine days later, phthalein test, 70 per cent. The urine showed a faint trace of albumin but no casts. Eleven days after inoculation the left kidney was removed. Weight 1,660 grams. Death on the twelfth day.

Left kidney: gross examination. The organ is swollen. The cut surface is succulent and markedly cloudy.

Microscopic examination. The fresh specimen shows an extremely large number of dark tubules filled with albuminous granules. Paraffin preparations show more than half of the tubules dark, due to the presence of deeply stained granules within the cytoplasm of the cells. The rods of Heidenhain are present in the basal part of the cells between the granules. The cytoplasm of the cells in all the tubules is intact. No fat is present.

Experiment 24.—Rabbit; male; weight 1,555 grams. The left kidney was removed. An abscess developed at the site of the operation. Killed 18 days after nephrectomy.

Right kidney. The organ is swollen. The cut surface is succulent and extremely cloudy.

Microscopic examination. Fresh preparations show extremely numerous dark tubules Paraffin sections show most of the tubules dark, due to the presence of deeply stained granules. Sections stained for fat show none present.

Experiment 25.—Rabbit; female; weight 2,210 grams. Inoculated with *B. proteus*. A large abscess developed. Twelve days later the animal died. Weight 1,775 grams.

Kidneys: gross examination. The organs are markedly swollen. The cut surface is succulent and distinctly cloudy.

Microscopic examination. The fresh specimen shows a large number of dark tubules which are filled with albuminous granules. Paraffin sections show the cells of a majority of the tubules filled with albuminous granules. The rods of Heidenhain are not present. (The kidneys were not obtained until some time postmortem.) The cytoplasm of the cells appears intact in all the tubules.

Experiment 26.—Rabbit; female; weight 1,730 grams. Inoculated subcutaneously with

pasteurella. An abscess resulted. Twenty-three days later the animal was killed. Weight 1,105 grams.

Kidney: gross examination. The organ is not swollen. The cut surface is moist and moderately cloudy.

Microscopic examination. There are numerous dark tubules filled with albuminous granules. Paraffin sections show the greater number of the convoluted tubules dark and filled with granules. These granules tend to be confined to the basal portion of the cells. The rods are prominent between the granules. The cells of most of the tubules are swollen so that no lumen is visible. There are a few tubules, however, which have distinct lumina and these often contain albuminous material. No fat could be demonstrated in frozen sections.

Experiment 27.—Rabbit; male; weight 1,850 grams. The animal was recovering from a pasteurella abscess. It was inoculated with pasteurella. Fourteen days later it was killed. Weight 1,565 grams.

Kidney: gross examination. The organ is moderately swollen. The cut surface is slightly cloudy and succulent.

Microscopic examination. The fresh preparation shows numerous dark tubules which are filled with albuminous granules. Paraffin sections show most of the tubules dark, due to the presence of darkly staining granules within the cells. In a few tubules they are arranged in rows between the rods of Heidenhain. The rods of Heidenhain are present in places between the granules and are very prominent in the less granular tubules. There are some dilated tubules which contain casts and albuminous material. No fat could be demonstrated.

Experiment 28.—Rabbit; male; weight 1,945 grams. The urine was normal. Phenolsulphonephthalein, 81 per cent. Inoculated subcutaneously with *B. proteus.* An abscess developed. Nine days later the urine was normal and the phenolsulphonephthalein test was about the same. Eleven days after inoculation the animal was killed.

Kidneys: gross examination. The organs are markedly swollen. The cut surfaces are definitely cloudy and succulent.

Microscopic examination. The fresh preparation shows a large number of dark tubules. Paraffin sections show that almost all the tubules are dark. The granules vary considerably in size. The rods are well preserved and prominent.

Experiment 29.—Rabbit; male. Inoculated with pasteurella. A large subcutaneous abscess developed. Three weeks after inoculation the animal was killed.

Kidney: gross examination. No swelling is present. The cut surface is distinctly cloudy.

Microscopic examination. The fresh preparation shows numerous dark tubules with albuminous granules. Paraffin preparations show almost all the convoluted tubules dark, due to the presence of numerous darkly staining granules within the cells. These granules vary in size. Some are half the size of the nucleus. The rods show in the basal part of the tubules between the granules. The cytoplasm of the cells of all the tubules is intact. No fat is present.

Experiment 30.—Rabbit; male; weight 1,560 grams. Phenolsulphonephthalein test, 75 per cent. Inoculated subcutaneously with *B. proteus.* An abscess developed. Thirteen days later phthalein test was 85 per cent. The urine showed a trace of albumin but no casts. The animal died shortly after the phthalein test was made.

Kidneys: gross examination. The organs are not swollen. The cut surfaces are slightly pale. The inner part of the medulla is extremely light colored.

Microscopic examination. The fresh specimen shows numerous dark tubules. Paraffin sections show that almost every convoluted tubule is dark. The rods are distinctly visible in most of the tubules. No fat is present.

Experiment 31.—Rabbit; male; weight 2,140 grams. Inoculated subcutaneously with pasteurella. A large abscess resulted. Twelve days later the animal died. Weight 1,365 grams.

Kidneys: gross examination. There is no swelling present. The cut surface is moist and definitely cloudy.

Microscopic examination. The fresh specimen shows numerous dark tubules filled with albuminous granules. Paraffin sections show almost all the tubules dark, due to the presence of darkly staining granules. In but few tubules are they confined to the basal

part of the cells. The rods are present in the basal part of the cells between the granules. The cells of the tubules are swollen so as to obliterate the lumina.

Zenker fixation. Paraffin preparations show no dark tubules. The granules are absent with the exception of a few scattered ones in some of the tubules. These do not stain intensely with iron-hematoxylin.

Experiment 32.—Rabbit; male; weight 2,260 grams. The animal was inoculated subcutaneously with pasteurella. A large abscess resulted. Twelve days later the left kidney was removed. Weight 1,690 grams. Three weeks after the inoculation the animal was killed. Weight 1,325 grams.

Left kidney: gross examination. The organ is swollen. The cut surface is moist and slightly cloudy.

Microscopic examination. The fresh preparation shows numerous dark tubules filled with albuminous granules. In some of the cells the granules are as large as the nucleus. There are a few granules which do not burst upon the addition of alkali but dissolve

Fig. 4.—Exp. 32. Kidney from a case of chronic infection. Zenker-formol. Iron-hematoxylin. There is a very marked increase in the number of dark tubules. Photomicrograph. X 80.

gradually. These are less refractive than the other granules. Paraffin preparations show the greater number of tubules to be extremely dark, due to the accumulation of darkly staining granules within the cells (Fig. 4). In some tubules these granules are immense, being as large as the nucleus (Figs. 5 and 6). Most of the granules are spherical, but some are oval and others spindle-shaped. The rods are distinctly visible in places between the granules. The cells of the tubules are swollen so that the lumina are almost all obliterated. No fat is present.

Right kidney: gross examination. The organ is swollen. The cut surface is extremely cloudy and succulent.

Microscopic examination. There are more dark tubules than in the left kidney and the granules are larger. Otherwise the kidneys are the same. There is no fat present.

Zenker fixation (right kidney). Paraffin sections. The granules are absent except in relatively few tubules. There are numerous light spaces in the cytoplasm of the cells. These are probably the spaces from which the granules have been dissolved out.

Experiments 33-50.—In a large number of experiments the rabbits died within one to

four days after the inoculation. The pictures presented by the kidneys in these cases were almost absolutely uniform and may all be described together. Grossly, the kidney is swollen. The cut surface is succulent but not cloudy. The inner part of the medulla is often extremely light colored. Microscopically, the fresh tissue shows no dark tubules. There is often fat present in the form of small droplets. Paraffin preparations show no dark tubules. There are usually a few granules in some of the tubules. There are usually some tubules in which the cells are disintegrated. The lumina of these tubules are wide and contain albuminous material or casts. The rods are absent or if present at all may be made out in only a part of the tubules. There are usually a number of small fat droplets in the collecting tubules and in many of the convoluted tubules near the medulla.

It will be seen from the foregoing that the experiments on infection may be divided into two groups: (1) a chronic or subacute type in which the rabbits lived more than a week; (2) an acute type in which the animals died within the first few days.

Fig. 5.—Exp. 32. Small area from Fig. 4. The granules are very large and occupy the greater part of the cells. Photomicrograph. X 500.

The characteristic picture found in kidneys of the first group of animals (Exps. 15 to 32) is as follows:

Gross examination. The gross picture is variable. In some cases the cortex is distinctly cloudy and swollen, but in other cases these features are barely noticeable. The medulla rarely shows any change. The cut surface is moist.

Microscopic examination (fresh). The fresh crushed specimen shows, under low power, a large number of dark tubules. These are seen upon high magnification to be filled with weakly refractive, definitely circumscribed granules, which almost conceal the nuclei. Upon addition of alkali these granules disappear like the bursting of a bubble, leaving the tubules light and the nuclei

clearly visible. These granules are also present in the light tubules, but they are fewer and scattered. In the more pronounced cases almost every convoluted tubule may be dark. In these instances some tubules contain immense granules, almost as large as the nucleus. These also burst upon the addition of alkali. No fatty granules are present.

In severe cases there appears another type of granule. This is less refractive than the former type and dissolves gradually in alkali.

Fixed and stained preparations. Hematoxylin and eosin. The cells of the convoluted tubules appear swollen, their inner borders projecting into and often obliterating the lumina. The cytoplasm stains well and, with low power,

Fig. 6.—Exp. 32. Drawing of small area from Fig. 4, showing details of structure. X 660.

seems to be perfectly homogeneous. Upon examination with oil immersion, however, the granules can be seen as bright red staining bodies.

Iron-hematoxylin. The cells of the greater number of convoluted tubules are seen to be filled with intensely staining granules. These are, as a rule, more numerous in the basal portion where they often show a tendency to be arranged in rows. In the inner portion of the cells they are irregularly scattered through the cytoplasm. In some tubules the granules are distributed uniformly through the whole cell. As described in the fresh specimen, the size of the granules varies. In the more pronounced cases they are very irregular in size, some being as large as the nucleus. Most of the granules appear spherical, but there are also oval and spindle-shaped ones in some of the tubules. The rods of Heidenhain may be seen in places between the masses of granules and are very prominent in the less granular tubules. There is no fat present.

The characteristic picture of kidneys of the second group of animals (Exps. 35-50) is as follows:

Gross examination. The gross, as a rule, shows no change, but sometimes the organ is succulent and swollen. In some cases the inner part of the medulla is extremely pale.

Microscopic examination. In fresh preparations there are no dark tubules. There are generally a few albuminous granules, however, scattered through the tubules. There are often scattered albuminous granules which do not dissolve upon the addition of acid or alkali. These are often particularly numerous in the glomeruli.

Fixed and stained preparations. Hematoxylin and eosin. There is always a varying number of tubules with wide lumina containing albuminous material and sloughed off portions of cells. The inner borders of the cells in many of the tubules contain vacuoles. These often give a ragged appearance to the cells.

In fixed material stained with iron-hematoxylin, the dark tubules are found to be entirely absent. A varying number of tubules contain small sparsely scattered granules which do not often show any tendency to be arranged in rows. The rods are absent as a rule. In some of the kidneys they are present in a few of the tubules, but absent in the greater number. In these acute cases there are often darkly staining bodies in the glomeruli. There is considerable fat in the form of fine granules in the collecting tubules and in the convoluted tubules near the medulla.

The most striking difference between the kidneys of acute and those of chronic infections is the almost complete absence of albuminous granules in the former and their presence in enormous numbers in the latter.

In the acute cases the renal tubules always show much more marked signs of injury than they do in the chronic cases. In the chronic group the phthalein test remains usually about normal and the cell structure is well preserved. These facts suggest that the marked increase of albuminous granules is not a degenerative change.

The number of dark tubules is greatest in those cases where there has been an extreme but very gradual loss of weight (Exps. 29-32). The albuminous granules in the dark tubules in the chronic cases are larger and more numerous than those in the dark tubules of a normal kidney. In some cases they attain an enormous size, sometimes being as large as the nucleus. The large granules are probably formed by fusion of smaller granules, since the granules are fewer in cells containing the large types.

The albuminous granules in the chronic cases closely resemble the granules of the dark tubules of the normal kidney. They burst when sodium hydroxid is applied, and they disappear in acetic acid solutions. They are fixed and stained by the same procedures. They are entirely different from the hyaline granules often seen in human nephritic kidneys. They are evidently thin-walled vesicles filled with fluid and not solid bodies.

In very severe infections one often sees granules that are less refractive than those described above. These granules disappear gradually in sodium hydroxid. They do not burst in this solution.

There is no fat present in the chronic cases. There is no evidence that

this increase of albuminous granules is in any way related to the formation of fat.

Fat droplets appear in the acute cases. It seems that toxic substances which cause the formation of fat droplets at the same time destroy the albuminous granules.

The gross changes in the kidneys of the chronic cases usually vary in degree, depending upon the number of albuminous granules present. Sometimes, however, there are many more granules present than is suggested by the gross picture. The kidneys are sometimes very opaque, but a typical "cooked appearance" was never obtained.

EFFECT OF ACUTE INFECTIONS UPON THE KIDNEYS OF CHRONIC CASES.

It has already been shown (Exps. 33-50) that acute infections cause the disappearance of the albuminous granules of normal kidneys. Exps. 51 and 52 were made to determine the effect of acute infections upon the kidneys of chronic cases which contain enormous numbers of albuminous granules.

Experiment 51.—Rabbit; male; weight 2,395 grams. The animal was inoculated with proteus. Four days later the left kidney was removed. Weight 2,175 grams. The animal was then reinoculated with a large dose of proteus intended to be rapidly fatal. Death the next day. Weight 2,080 grams.

Left kidney: gross examination. The organ is swollen. There is no cloudiness present.

Microscopic examination. Dark tubules are present in the fresh preparation. Paraffin sections show about half of the tubules moderately dark. These tubules contain fewer dark granules than the dark tubules of more severe infections. The rods are well preserved. The cytoplasm of the cells is intact in all of the tubules.

Right kidney: gross examination. The organ is swollen. The cut surface is succulent but not cloudy. The inner part of the medulla, however, is extremely pale.

Microscopic examination. The fresh preparation shows no dark tubules although there are a few albuminous granules in some of the tubules. Paraffin preparations show no dark tubules. The cytoplasm of the cells of many of the tubules is fragmented. The lumina of these tubules are wide and often contain albuminous material. The rods are well preserved in only a few tubules.

Experiment 52.—Rabbit; male; weight 1,890 grams. Phenolsulphonephthalein test, 68 per cent. The animal was inoculated subcutaneously with *B. proteus*. Thirteen days later the phthalein test was 70 per cent. Weight 1,150 grams. The animal was then given a very large dose of *B. proteus*. Death the next day.

Kidneys: gross examination. The kidneys are swollen. The cut surfaces are succulent and moderately cloudy.

Microscopic examination. There are no dark tubules in the fresh preparation. Paraffin sections show no dark tubules. There are a few scattered darkly staining granules in some of the tubules, however. The rods of Heidenhain are present in most of the tubules. A few of the tubules are dilated and contain casts and albuminous material within their lumina. The cells of a few tubules are fragmented on their inner borders. Sections stained for fat show a considerable quantity present in the convoluted and collecting tubules in the form of fine granules.

ETIOLOGY AND ULTIMATE SIGNIFICANCE OF CLOUDY SWELLING.

Various interpretations of cloudy swelling are to be found in the literature. The ideas may be grouped as follows:

1. Excessive functional activity. This was the belief of Virchow. He regarded the granules as nutritive material taken into the cells but not assimilated. Birch-Hirschfeld recognized one kind of cloudy swelling of this type,

and another in which the granules represent surplus waste material. Albrecht and Landsteiner interpret some cases of cloudy swelling (compensatory) as due to increased physiological activity. Adami believes that the granules are due to excessive and prolonged stimulation of the cells.

2. Coagulation or precipitation theory. Cohnheim, Rindfleisch and Klebs believed that the granules are formed from the coagulation of fluid albumins in the cells. Klebs and Fischer[22] explained the coagulation as due to acid formation in the cytoplasm.

3. Degeneration theory. Many authors regard cloudy swelling as essentially a degenerative process. It is explained as due to decreased oxidation (Benario), to poisonous substances (Ribbert, Schilling), and to decreased nutrition (Thoma). Others who subscribe to this view are Birch-Hirschfeld, Albrecht (one type of cloudy swelling), Volhard and Fahr. Some observers emphasize the intimate relation between cloudy swelling and fatty metamorphosis (Benario, von Recklinghausen, Ziegler, Birch-Hirschfeld, Ribbert, Rindfleisch, Wells,[24] Thoma, and Adami). Many believe that cloudy swelling is a process that may readily pass into definite cell degeneration and necrosis (Virchow, Cohnheim, Ziegler, Ribbert, Rindfleisch, Thoma, Adami, Volhard and Fahr).

4. Emulsification theory. Albrecht believes that one form of cloudy swelling represents an emulsification of the cytoplasm, due to imbibition of fluid and separation of lipoidal substances. Von Gierke and Anitschkow[25] accept this interpretation in a modified form.

Albrecht recognized three fundamental types of cloudy swelling: emulsification, excessive function, and disintegration. Some other observers also believe that there is more than one fundamental process concerned.

Nearly all the authors mentioned above have worked exclusively with human cloudy swelling. It is easily possible that some of the processes involved here are different from those concerned in the rabbit kidney.

My observations indicate that the cloudy swelling occurring in tartrate nephritis is due to edema, anemia, fat, tissue disintegration, etc., and is not associated at all with albuminous granules.

But the cloudy swelling of the kidney following chronic infections is evidently due in part to a marked increase of albuminous granules in the cells. This type of cloudy swelling is probably not a degenerative process, since the phthalein test nearly always shows normal functional activity (Exps. 17, 23, 28, 30). It seems to be a physiological response to an increased quantity of protein waste products in the blood.

DISCUSSION.

Much of the confusion in the diagnosis of cloudy swelling is due to lack of agreement among pathologists as to what constitutes the gross picture. If we make the diagnosis only when the organ looks "cooked," then we shall almost invariably find this gross picture associated with a marked increase of albuminous granules in the cells. If we take the position that this classical picture and this alone constitutes cloudy swelling, then the condition becomes very rare. All the milder degrees of this same process would be excluded, since the typical "cooked" appearance occurs only when the cells are crowded with al-

buminous granules. This definition of cloudy swelling would also exclude all the experimental lesions described in this paper, since none of them had the typical "cooked" appearance.

The average pathologist often makes a diagnosis of cloudy swelling in an organ that is merely turbid and swollen, in the absence of the typical "cooked" appearance, and without a microscopic examination of the fresh tissue. If the term is used in this sense then cloudy swelling becomes one of the most frequent of autopsy findings. Cloudiness and swelling in an organ may be due to edema, anemia, fat, albuminous granules, tissue disintegration, etc. This use of the term obviously makes it include several distinct pathologic phenomena.

In my experiments the kidneys of animals injected with tartrates and autolyzed liver tissue were often swollen and cloudy. These changes in the gross appearances were apparently due to edema, anemia, tissue disintegration, etc.

The kidneys from the cases of chronic infections were also often cloudy and swollen, but here the gross changes were due mainly to an enormous increase of albuminous granules.

In my opinion the term cloudy swelling should be dropped out of pathologic literature. The classical cloudy swelling is merely an intense accumulation of albuminous granules in the cells and is more clearly described as such. If the term is to be used to describe a turbid swollen organ, it becomes worse than useless, since it groups together several unrelated pathologic phenomena.

There would be less confusion if the term cloudy swelling were discarded entirely and the several phenomena which produce this appearance were considered separately. Thus, instead of studying cloudy swelling, we should study the increase of albuminous granules, edema, fatty metamorphosis, emulsification of the cytoplasm, etc.

SUMMARY.

The normal rabbit kidney always contains, in the convoluted tubules, coarse albuminous granules. Usually these granules are so numerous in a few tubules that these appear dark in the fresh tissue. The granules are apparently thin-walled vesicles filled with fluid. They are best fixed by solutions containing formalin. They are not fixed in solutions containing acetic acid.

When one kidney is removed the dark tubules are increased in the opposite kidney during the first twenty-four or forty-eight hours, but the increase of albuminous granules is not sufficient to cause any definite change in the gross appearance of the kidney.

Subcutaneous injections of tartrates produce a swollen, cloudy kidney, but there is no increase of albuminous granules. The cloudiness and swelling are apparently due to edema, anemia, tissue disintegration, etc.

Intraperitoneal injections of autolyzed liver tissue produce a markedly cloudy and swollen kidney. The albuminous granules disappear entirely. The gross changes are apparently due to the same factors concerned in the tartrate experiments.

Chronic suppurative processes attended with marked emaciation cause an enormous increase of albuminous granules in the kidneys. These granules are

often larger than the normal and irregular in shape, but they seem to have the same chemical composition.

Acute toxemias cause a rapid disappearance of the normal albuminous granules.

An acute toxemia superimposed upon a chronic suppurative process causes a disappearance of the albuminous granules.

Kidneys which show an enormous increase of albuminous granules usually give a normal phthalein output, and the cells are usually intact. This form of cloudy swelling is therefore probably not a degenerative change, but a physiological response to an increase of protein waste products in the blood.

There is no relation between the formation of albuminous granules and fatty metamorphosis.

It is suggested that the term cloudy swelling be discarded and that the several processes producing this appearance be considered separately.

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PROGRESSIVE LENTICULAR DEGENERATION*

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In 1912 Kinnier Wilson[1] described in detail lenticular disease and reported several cases with necropsies. During the last three years several observers have expressed their views as to the exact function of the nuclei of the corpus striatum. Mingazzi[2] has referred to its function in relation to the motor disorders and sensory accompaniments. He also states that should four-fifths of the left nucleus be involved that dysarthria might occur. Stocker,[3] in 1913, reported as a case of lenticular disease, one in which the external ocular muscles were involved in addition to the cardinal symptoms as described by Wilson, still the tremor was only slight and inconstant. Autopsy showed, however, a lobular atrophy of the liver and softening of both corpus striatum. He raises the question of differential diagnosis between lenticular degeneration and multiple sclerosis, diffuse sclerosis, senile and juvenile paralysis agitans. H. Oppenheim,[4] in 1914, described three cases which he classed as cases of pseudosclerosis. All his cases had a constant general body tremor, disturbances in speech and emotional instability. His feeling was that they were closely allied to Wilson's progressive lenticular degeneration, but he did not refer to the fact that in cases heretofore described as pseudosclerosis there was no disturbance in the liver whereas in Wilson's cases the liver showed, invariably, a toxic cirrhosis. A case report, described by Mills[5] late in 1914 revealed at necropsy an atrophy of a large part of both caudate nuclei and a symmetrical sclerosis of the anterior portion of each lenticular nucleus. In his discussion he refers especially to the symptoms referable to the caudate nucleus such as hypertonicity and paresis of the affective motor apparatus, painful emotional expressions and various other symptoms probably in relation to the cortico-autonomic nervous system. In addition he relates its possible effect upon the vasomotor system, causing disturbances in temperature, pulse and glandular activity. In his case, as in the one here to be reported, there was also infringement upon the internal capsule. There is little doubt, however, but that more of the symptoms were due to extra-pyramidal lesions and should contribute a few facts as to the pathogenesis of extra-pyramidal disorders. Cadwalader[6] has reported a case with autopsy at which time a bilateral lesion of the lenticular nuclei and a cirrhotic liver were found.

It is because of the great interest that is being demonstrated in relation to the functions of the lenticular nucleus and the symptoms produced by its disease that it was deemed opportune to report this interesting case of lenticular disease with the autopsy findings.

Case.—H. H., female, age 19, no occupation, single.

Family History.—It was considered negative for two generations. The father died of pulmonary tuberculosis and the mother from heart disease. One

*From the Clinico-pathological Department, Rhode Island State Hospital for Insane. Presented before the New York Neurological Society, January 4, 1916.

brother and two sisters are alive and well. No history of nervous or mental disease as far as could be ascertained.

Personal History.—The patient was the fourth in a family of six children. She had measles as a child. She attended school from five until fourteen, reaching the sixth grade. Her reason for not reaching a higher grade was that she had to remain at home to take care of her mother. During the summer of 1912 she was employed in a rubber factory, also in a stocking factory for two weeks. She left the first place because she was nervous. She was discharged from the second position because of pediculæ capitis. The patient never menstruated. She had no serious illness until the present.

Present Condition.—Her troubles appear to date back four years when she began to have epileptoid seizures, the description of which it has been hard to obtain. The patient called them convulsions. She had three at or about the time of the death of her mother in 1912. Since that time she has been "nervous," suffering from a coarse tremor of the hands and legs which caused her great difficulty in coordinating. During the month of May, 1913, she became so incapacitated that she was admitted to the Rhode Island General Hospital.

*Abstract from the Rhode Island General Hospital.**—The patient was well nourished. She had no disturbance in her internal viscera as far as could be ascertained. She had no pupillary disorder. The superficial abdominal reflexes were absent. The knee-jerks were exaggerated unequally, the right greater than the left. There was a suggestion of a Babinski sign on the left side. The gait was somewhat spastic. There was no disturbance in sensation. The extremities evinced evidence of a poor circulatory condition. The most characteristic feature of the disorder as observed in the hospital was the coarse incoordinate tremor of both arms, her refusal to talk and her refusal to eat at times. While in the hospital, on July 25, she had her first epileptoid seizure, resembling true epilepsy but thought to be hysterical. She had several of these attacks while under observation but they were finally thought not to be especially characteristic. During her stay, from May to October, her condition became progressively worse with the addition of emotional instability and aphonia. Dementia præcox, hysteria and multiple sclerosis were considered. She was discharged October 14 to go to the State Almshouse.

*Abstract for the State Almshouse.**—The patient was described as being excited, hilarious and noisy. She made attempts to escape. She was filthy, spat at people; she was violent and kicked the nurses. She was resentful. Observations indicated that the question of epilepsy had not been satisfactorily settled; she did not have any seizures and with the exception of fits of temper and destructiveness as well as an uncontrollable rage, there was nothing to suggest epilepsy. Most of the time she was in a state of great emotional and mental excitement with psychomotor unrest. She was resistive at times and again quiet. She sang, hummed, cried and swore, using much vulgarity colored with sexual thoughts. Observations pointed to the fact that she had had some spinal cord disorder somewhat similar to anterior poliomyelitis. She was discharged Feb-

*We wish to express our appreciation to Dr. John M. Peters of the Rhode Island Hospital and to Dr. Henry A. Jones of the State Almshouse for their kindness in furnishing us with abstracts.

ruary 5, 1914, as a case of hysterical insanity and transferred to the State Hospital for the Insane.

At the *State Hospital* for the *Insane* her physical condition was characterized by choreiform movements of the extremities, unsteadiness in gait and a profuse, loquacious stream of thought without any defects in memory, disturbance of thought or appearance of trends. She was excited, talkative, fearful and destructive. There were no delusions nor hallucinations. She was perfectly well oriented for time, place and person. Consciousness showed no disturbance and there was no evidence of mental confusion. Her attention was readily gained but poorly held owing to her distractability. Her trend of thought was spontaneous and coherent with occasional periods of distractability. Intellectually she appeared to be somewhat inferior for a girl of her age.

She was placed in the continuous bath several times during her residence

Fig. 1.—M. H. Seven weeks before death. Characteristic contractures, hyperextension of head, dropped jaw; position indicates condition of spasticity.

owing to her marked psychomotor unrest. From April 26, 1914, to January 14, 1915, the patient showed a gradual decline, physically. She ran a constant temperature of 103 degrees, cried a great deal, had little to say and at times she laughed without apparent cause. By this time there had been distinct changes in her physical condition. Her limbs had become contracted. A general body and limb tremor manifested itself markedly. It was necessary to tube-feed her to maintain nourishment. She continued in this condition and was seen by one of us (F) on February 2, 1915.

Neurological Examination at that time presented the following: The patient was very thin and showed strikingly, at first glance, a dropped jaw with a masked expression of the face. She was easily aroused to emotion. She did not respond to simple or complex commands. Contractures and fixation of all the joints, small and large were manifest (see Fig. 1). She was running a constant temperature of 103 degrees.

Contractures.—Evidently all her joints were fixed and rigid. There was some pain upon any attempt to straighten them out. Her head and neck were fixed and hyperextended. Her jaw was dropped and she could not hold it up, she could not show her teeth by gritting. The fingers were flexed, thumbs turned into the palms of the hands, wrists flexed and averted so that the palms looked outward (see Fig. 1). The arms were abducted, the forearms abducted, due to flexing and fixation at the elbow. The legs and thighs were flexed and fixed. The legs were also drawn up on the abdomen. The feet were turned in and a dorsal extension of both toes was present. Her entire musculature was firm and hypertonic.

Tremors.—There was a general body tremor involving also the tongue and jaw. This tremor was increased by excitement and also when she tried to hold herself up in the bed, under effort. It was quite noticeable when the photograph was taken. It was regular in constancy, but varied in tone according to emotion. When she made an effort there appeared to be a spasm of all her flexor muscles. There were no fibrillary twitchings and no typical athetoid or choreiform movements.

She could not eat due to the spasm of the muscles of mastication (dysphagia). She could not talk due to spasms of the muscles of articulation (anarthria), although at times she could pour forth a most profuse vulgarity (profanity can hardly be called a genuine form of speech, there is no specific appropriateness; they are little more than animal outcries; as such they might survive the destruction of that duty of producing thought with selected words).

There were no cranial nerves involved as far as cooperation would allow. The pupils were equal and regular in outline. They reacted well to light and to distance. The muscular movements of the eyes showed no disorder. The optic discs were negative. There was no nystagmus and no evidence of labyrinthine or cerebellar disease.

She had never vomited nor shown any evidence of heart involvement or autonomic nervous phenomena. At no time had there ever been noted any evidence of liver disorder and physical examination showed no enlargement or atrophy by percussion and palpation. She was unable to protrude her tongue which might have been due to spasticity or to paralysis.

The superficial reflexes of the upper extremities were diminished. The abdominal and epigastric reflexes were absent. The knee-jerks were diminished probably due to the flexion and fixation of the abductor muscles. The right great toe suggested dorsal flexion. There was also an atypical Oppenheim and Gordon great toe response. The left great toe presented a plantar flexion. She could not walk, sit up or coordinate to any degree for the tests for gait. Her attitude and posture were quite characteristic (see Fig. 1).

There was no evidence of sensory disturbances.

Physical examination of the viscera evinced tuberculous processes in both lungs. No tubercle bacilli found in sputum. The von Pirquet test was not made.

The spinal fluid analysis evinced two cells per cubic mm. The Wassermann reaction on both the blood and spinal fluid was negative. Lange's colloidal gold reaction was negative. The globulin reactions by the technic of Nonne, Noguchi, Ross-Jones and the combined method gave negative findings.

At this time the diagnosis had resolved itself into either juvenile paralysis agitans or Wilson's progressive lenticular degeneration. The presence of pyramidal tract symptoms was rather in favor of a central disorder with infringement upon the internal capsule so that at that time a tentative diagnosis of progressive lenticular degeneration was made.

During the next month she was seen several times and further observation led us to conclude the case to be one of actual brain disease with the lesion in the region of the lenticular nucleus. She died March 4, 1915, and the necropsy verified the diagnosis by the finding of a typical cirrhotic liver and a degenerated left lenticular nucleus.

To Recapitulate the Clinical Findings.—A young woman of nineteen years whose physical development was normal and whose mental development was probably one of inferiority (constitutional inferiority) becomes "nervous" at the age of 15 years and has what is called a "convulsion" following which there is noted a tremulousness of the extremities and a later definite involvement of the central nervous system associated with changes in motion and affectivity. Dysarthria and dysphagia developed and a spastic emotional state soon became prominent. Along with all these phenomena of muscular hypertonicity there was, from the beginning of the disease, a coarse rhythmical tremor of the entire body. The neurological examination did not reveal any disturbance of the cranial nerves, there was no evidence of sensory disorder and with the exception of a dorsal flexion of the right great toe there were no signs of a disturbance of the pyramidal tract system, yet, the masked expression, dysarthria, dysphagia and increased motor tonicity were present. Although, the face was spastic and the mouth wide open and to all appearances in a severe grade of mental deterioration, when attempts were made to move her, she watched every movement and assumed more markedly the painful emotional expressions as described by Wilson and Mills. There was no evidence of liver disorder and the temperature could be accounted for by the tubercular processes in both lungs.

Autopsy Findings.—The autopsy was performed four hours after death. A general observation of the body showed the prominence of contracturers of the extremities. She was greatly emaciated with trophic skin lesions and secondary infection.

Without going into detail regarding the body viscera* suffice it to be stated that areas of tubercular lesions, healed and active, were found in both lungs. The heart was normal. The spleen showed passive congestion and enlargement. There was no affection, grossly, of the kidneys or adrenals noteworthy. There was an infantile uterus and atrophic ovaries. The large intestines, especially the descending colon, showed many confluent tubercular ulcers, none having perforated, however. The thyroid was atrophic, no preservation of the thymus gland. The liver showed a typical nodular cirrhosis with a glossy capsule and which, upon cut section, present a multilobular cirrhosis.

Histopathological Symptom-Complex.—*Liver.*—The appearance of the liver was exceedingly striking and suggested immediately Laennec's cirrhosis. It was somewhat smaller than normal, yet in no way misshapen and weighed

*We wish to express our thanks to Dr. H. S. Bernstein for his report upon the abdominal and thoracic viscera.

1,030 grams. Its consistency was firm and cut on section with marked resistance. The external surface was smooth but gave one the feeling of slight elevations and shallow depressions as the fingers were passed over the surface. Upon cut section there were presented multiple lobulations, varying in size from that of a pin head to that of the little finger nail, varying in shade of color but in no way yellowish to indicate jaundice or green to indicate extravasation of bile. These lobulations were somewhat oval in shape and were separated from each other by strands of connective tissue varying in amount. The gall bladder showed no signs of disorder and there were no stones in the ducts. (See Fig. 2.)

The sections for microscopical examination were stained with hematin and eosin and toluidin blue. The most striking feature was the preservation of liver cells in certain areas, with the sudden appearance of degenerated areas, large in area compared to the normal liver cell areas, and in which fatty degeneration was prominent. Then, again, the appearance around these areas of connective

Fig. 2.—M. H. Cut section, showing the whole liver to be cirrhosed. The cirrhosis is multilobular, without being bile-stained and divided by connective-tissue hyperplasia.

tissue hyperplasia, varying in breadth and degree of fibroblastic activity with a moderate infiltration of round cells. Some of the sections indicated by the scarcity of round cells and the relatively few elongated nuclei of the fibroblasts undoubtedly were older processes. In these connective strands were occasionally seen groups of nuclei, arranged in somewhat parallel rows which might indicate new bile capillaries or hypertrophic bile ducts. In some places the strands of connective tissue appeared to invade the lobule and separate the liver cells into columns. There appeared also in certain portions, to be a connection between these so-called new bile capillaries and a column of liver cells. It has been suggested that they might be liver cells reverting to their primitive state rather than new capillaries. In the more degenerated areas necrosis to the extent of fatty infiltration and degeneration was very prominent (see Fig. 3) and the normal liver structure completely losing its cellular character. Throughout many of

the sections liver cells in the process of regeneration could be noted by the changes in the nuclei, double nuclei and a tendency to mitotic formation.

EXAMINATION OF THE BRAIN AND SPINAL CORD.—After the removal of the brain, a general inspection indicated that the left hemisphere was somewhat smaller than the right especially in the frontal region. There was no disturbance in the patterning of the cerebral convolutions. There were no palpable areas of softening or hardening indicating neoplastic growths. The brain was placed in hardening fluid (formalin) and cut when hardened.

Cutting each hemisphere as near as possible through the area suggested by Marie resulted in the following observations (see Fig. 4):

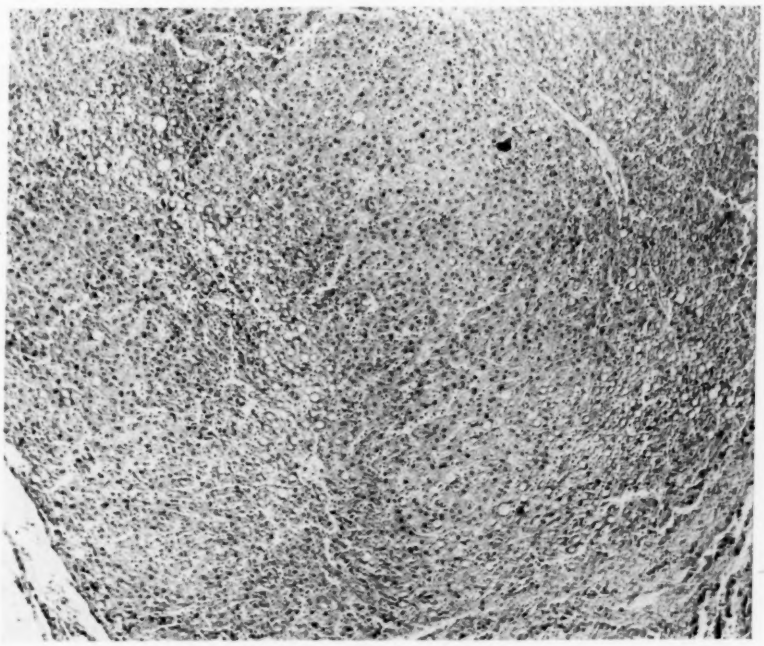

Fig. 3.—Low power of section of liver showing degenerated areas, connective-tissue hyperplasia, preservation of normal liver cells, and capillary formation.

It was first noticed that the markings characteristic of the corpus striatum were lacking on the left side and somewhat less marked on the right side. The left caudate nucleus appeared to be somewhat more shrunken than the right and there was a decided dilatation of the anterior horn of the left ventricle. The optic thalami showed no abnormality. The internal capsule on the left side appeared to be confluent with the tissue that evidently made up the area where the lenticular nucleus was primarily situated. There was no putamen or globus pallidus on the left side, as such and yet there were several small areas which suggested areas of necrosis. On the left side by palpation one noted that there was less resistance than on the right side and that to the finger touch it was soft, as if degenerated. There were no decided cavity formations. The only indication of an outer margin of the original lenticular nucleus was a small uneven broken strip less than a millimeter in width, separating the external

capsule from the degenerated lenticular nucleus. This small broken strip was very small in thickness as well as in breadth and about 1 cm. in length. The caudate nucleus was very much shrunken but still compared with the region of the lenticular nucleus it was well preserved and its fibres could be easily distinguished. A transverse section in the region of the corpus striatum of both hemispheres of that part of the brain removed in the initial cutting along the Marie line of selection, showed very plainly the shrunken caudate nucleus and the dilatation of the anterior horn of the left ventricle.

On the right side the markings were still somewhat distinct and the lenticular nucleus showed a preservation of both the putamen and the globus pallidus. The external capsule was present without disintegration and in the internal capsule, both in its anterior and posterior limbs, appeared to be normal.

The cortex and white matter of the brain in general showed no abnormality. The convolutions in the region of the Island of Reil showed no

Fig. 4.—M. H. Brain. Horizontal section showing almost complete destruction of the left lenticular nucleus and a partial destruction of the right lenticular nucleus; the caudate nucleus being much smaller on the left side with a dilatation of left ventricle.

obvious change or disorder. There was no evidence of internal hydrocephalus, no inflammation of the ependyma and there were no granulations in the fourth ventricle.

The spinal cord showed no abnormality in its macroscopical appearance. Sections of the cord from the cervical, dorsal and lumbar regions showed no disturbance in the markings as far as could be observed. No disturbance was noted in the meninges.

MICROSCOPICAL EXAMINATION.—*Spinal Cord.*—Histopathologically an examination of the various regions of the spinal cord showed no disorder in sections stained with hematoxylin and eosin and toluidin blue. It is unfortunate that in the primary fixing sections were not taken to examine by the Weigart and Marchi methods. With toluidin blue the sections showed

a fairly large number of cells and they seemed to have preserved their normal outline and appearance. The grouping appeared to be also normal. In the cervical region they stained in normal shape and yet a few were seen to be stained much more deeply but without any marked disturbance in the nuclei. Occasionally one would come across a cell which suggested the

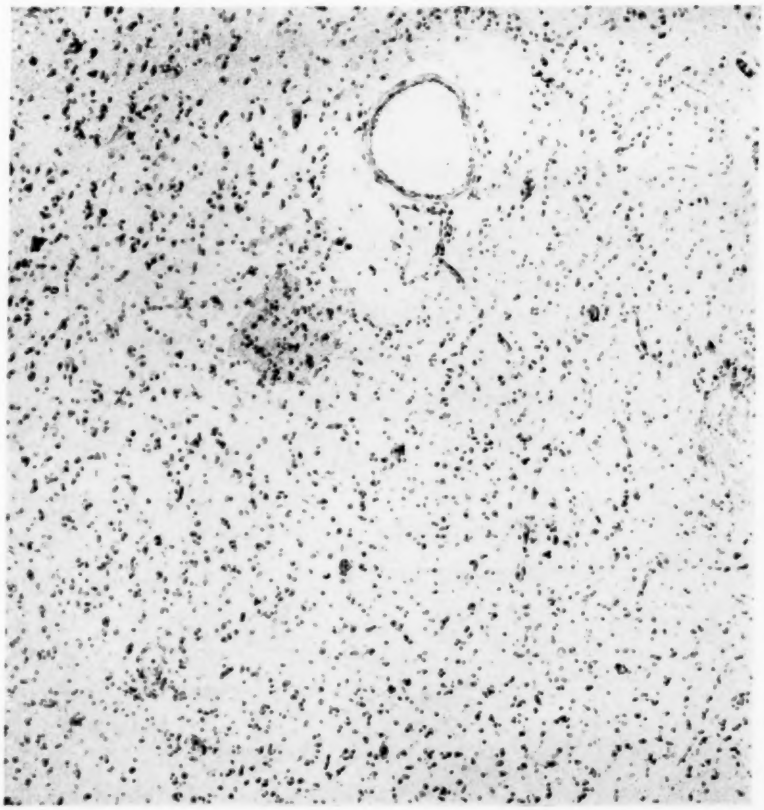

Fig. 5.—Low power of left lenticular nucleus, showing neuroglial increase, scarcity of nerve cells, no sclerosis of vessels but an enlargement or dilatation of Virchow-Robin space.

pyramidal cell in outline and yet was not unlike the pyramidal cells seen in terminal exhaustive infections.

Meninges.—Examination of the coverings of the brain and cord showed in no way abnormality. There was no thickening, no injection of the vessels, no lymphocytic or plasma cell infiltration.

Motor Cortex.—A serial section taken from the right motor cortex showed no abnormality in the arrangement of the cells in their various layers. There was no perivascular lymphocytic infiltration; there was no invasion of the cortex by plasma or round cells and there did not appear to be any inflammatory reaction as indicated by the increase in vascularity, new blood vessel formation or new lymphatic channels.

Sections taken from both the right and left lenticular nucleus evinced the following: *Left.*—The tissue seemed to be composed of a comparatively

capsule from the degenerated lenticular nucleus. This small broken strip was very small in thickness as well as in breadth and about 1 cm. in length. The caudate nucleus was very much shrunken but still compared with the region of the lenticular nucleus it was well preserved and its fibres could be easily distinguished. A transverse section in the region of the corpus striatum of both hemispheres of that part of the brain removed in the initial cutting along the Marie line of selection, showed very plainly the shrunken caudate nucleus and the dilatation of the anterior horn of the left ventricle.

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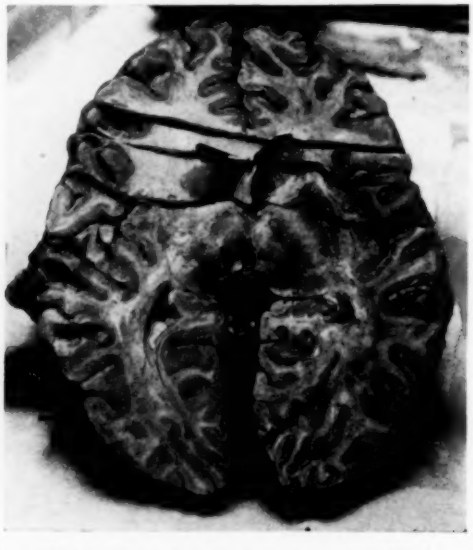

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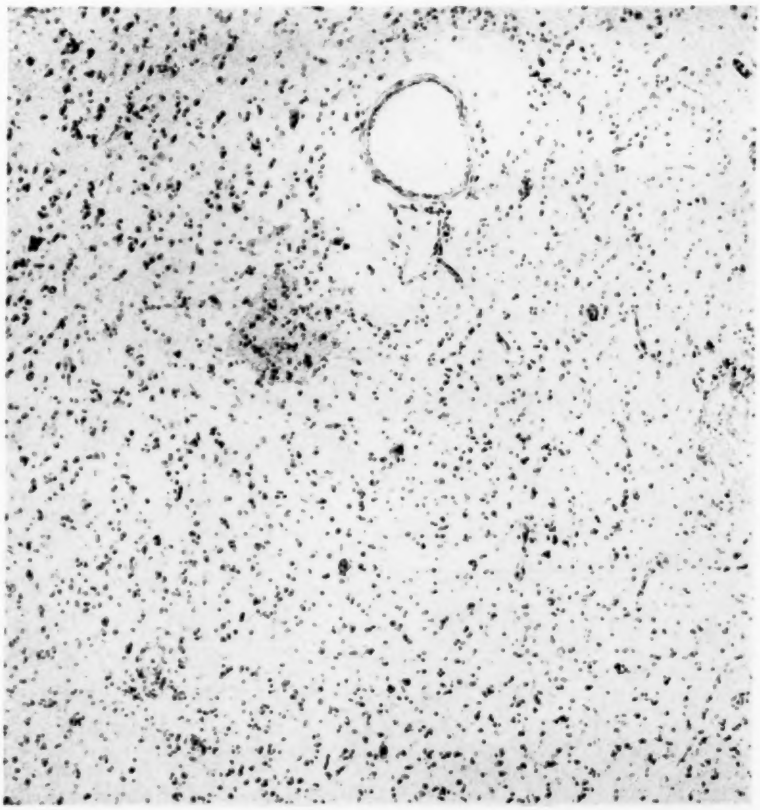

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Sections taken from both the right and left lenticular nucleus evinced the following: *Left.*—The tissue seemed to be composed of a comparatively

thick network neuroglial increase. In various fields it was noted that there were probably reactions such as is seen in satellitosis. The nerve fibers were few with only an occasional nerve cell, which cell was usually misshapen and the nucleus eccentrically situated, the cytoplasm deeply stained but not showing to any marked degree the granulation. The vessels were not scler-

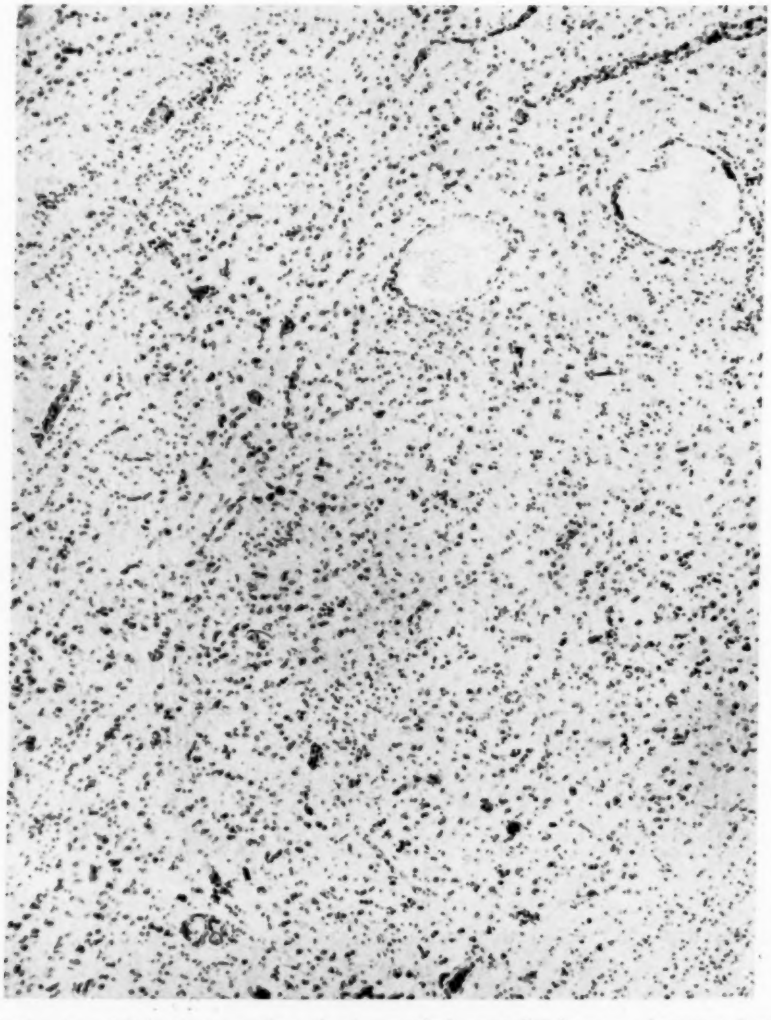

Fig. 6.—Section from left lenticular nucleus showing marked neuroglial increase, degenerated nerve cells in several stages, tendency towards the formation of satellitosis.

osed or occluded. It was not an uncommon observation to see a blood vessel with its thin coats lying in an open space, evidently an enlargement or dilatation of the Virchow-Robin space.

The most marked feature about the section from the left lenticular region was the few nerve cells not in the putamen and the globus pallidus. The caudate nucleus also showed some changes as the glial reaction found in the putamen. The internal capsule stained normally but did appear to

have a connective tissue reaction in the shape of an infiltration of round cells.

Right.—On the right side the reaction did not appear to have reached the stage as seen on the left. Still, there was a decided evidence of sclerotic changes as indicated by the neuroglial increase and the tendency to satellitosis. Under the high power the area seemed to be a mass of neuroglial overgrowth with new vessels and a scarcity of nerve cells, although more were found on this side than upon the left. The neuroglial tissue about the cells appeared

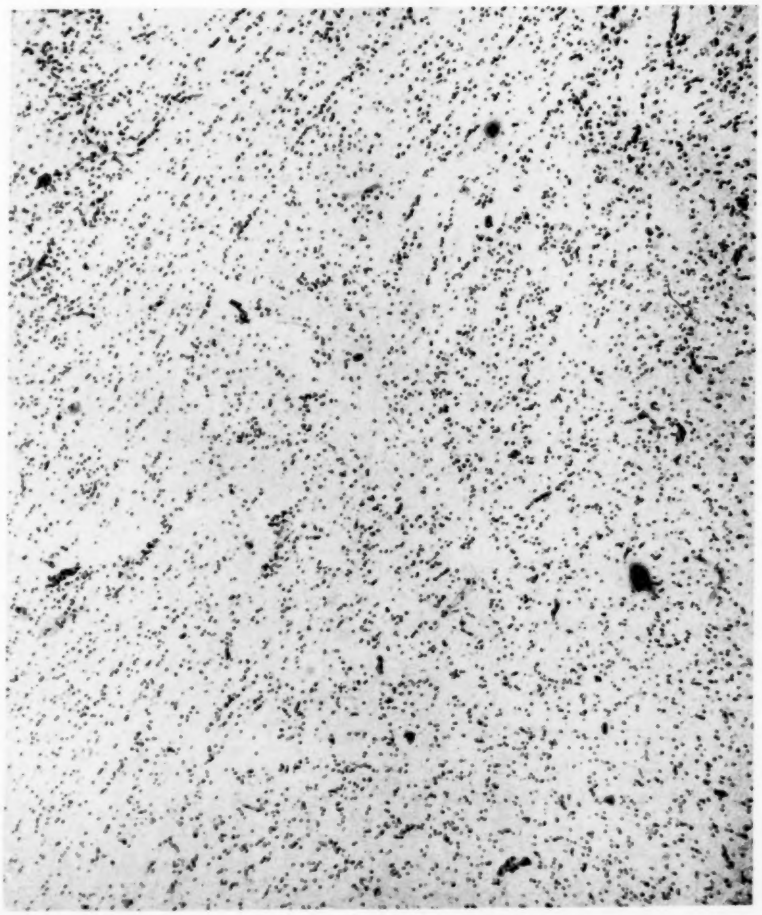

Fig. 7.—Sclerotic changes in the neuroglia are evident and in the upper left-hand corner is an indication of new vessel formation.

to be an active growth. The nerve cells in the region of the globus pallidus were shrunken or swollen and stained very deeply. In some fields the large pyramidal cells appeared to have taken on a coarsely granular appearance in their cytoplasm and had a deeply stained nucleus. About these cells there were frequently seen small elongated cells, probably the inflammatory neuroglial reaction. (Figs. 5, 6 and 7.)

To Recapitulate the Pathological Findings.—It is evident that the upper

motor neuron is in its entirety in no way involved in this disease except secondarily due to the infringement of the neoroglial overgrowth upon the internal capsule on the left side. There is no disturbance in the motor cortex and no disturbance in the cells of the anterior horns. The microscopical changes in the lenticular region appear to consist in a glial overgrowth which in some places has disintegration, in some places in the dense glial overgrowth an occasional pyramidal cell will make its appearance. There is occasionally seen a cellular infiltration suggesting a satellitosis. The vessels are not sclerosed or occluded but it is frequently noted that the Virchow-Robin space between the vessel and the surrounding tissue is dilated to a considerable degree. The pyramidal cells are scarce in number on both sides, more marked loss on the left side and those which are present show either a partial or complete cellular disintegrated as indicated by the cytoplasm and the nucleus. The entire reaction appears to be that of a replacement gliosis that has not gone on to complete disintegration with the formation of cavities although there are sections which indicate an advanced stage of necrosis.

Diagnosis.—Hughlings Jackson once said "positive signs could not be caused by negative lesions." The clinical symptoms, tremor, spasticity and emotional instability are essentially positive symptoms and one must therefore conclude that they are caused by a destructive lesion. In this case the destructive lesion appears to confine itself entirely to the lenticular nucleus and the caudate nucleus upon the left side, as far as the pathological examination has been able to point out. It is Wilson's view "that destructive lesions of the lenticulo-rubrospinal tract remove the normal inhibition or steadying influence which the corpus striatum is supposed to exercise upon the anterior horn cells." As a result of this removal the steady innervation of the anterior horn cells is impaired and the more the pyramidal tract is innervated the more obvious does the tremor become. This same explanation may be applied to the influence exerted by the cortical cells upon the pyramidal tract system and cause the development of the spasticity and hypertonicity.

The lenticular syndrome as described by Wilson is similar in many respects to that described in this case, yet, there is in addition an involvement of the right pyramidal tract system which might be interpreted as an extension of the gliotic process. However, it seems best to present the case clinically and pathologically, as one of progressive lenticular degeneration of Wilson's type.

We wish to express our indebtedness to Mr. William S. Dunn, of Loomis Laboratory, Cornell Medical University, for his work upon the photomicrographs.

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SPECIFIC PARENTERAL DIGESTION AND ITS RELATION TO THE PHENOMENA OF IMMUNITY AND ANAPHYLAXIS*

By J. Bronfenbrenner, Ph.D., Pittsburgh, Pa.

WHILE it is generally observed that repeated injections of toxins or toxic bacterial proteins render the organism resistant against even a multiple lethal dose of those toxic substances, experiments have shown that under certain conditions the reinjection of even absolutely inoffensive substances may produce very severe phenomena of intoxication and even death of experimental animals.

It is this exaggeration of toxicity which is the most striking phenomenon in anaphylaxis.

"Anaphylaxis is a reverse of vaccination; anaphylactic animals react to the second injection much more strongly than to the first, which is the more surprising because, in the majority of cases, the substances used are not toxic even in very large doses," wrote Besredka in 1908.

Where does this increased toxicity come from?

Since the substances injected may by themselves be inoffensive to normal animals, even in very large doses, it is evident that conditions in the body of anaphylactic animals must be responsible for the change in tolerance to protein.

Why is it then, that in the one case, the preliminary injection of protein raises the resistance of the animal to the subsequent introduction of the same protein, whereas in the other case it destroys even the natural tolerance of the animal, making it incomparably more vulnerable than before?

It is this *loss by the animal of its normal degree of immunity,* or resistance to the parenteral introduction of foreign proteins, which suggested to Richet the name of *"Anaphylactic"* for this state of hypersensitiveness of the experimental animal, following a preliminary inoculation with the foreign substance. The very name given to this phenomenon by Richet, who was one of the first investigators in this field, shows that the nature of the anaphylactic state was assumed to be quite the opposite of that of immunity, or heightened resistance, in spite of the fact that both conditions seem to be brought about in the experimental animal by a very similar procedure.

It is because the parenteral introduction of foreign protein may lead in one case to immunity, and in the other to hypersensitiveness, that some authors suggested as a working hypothesis, that the introduction of foreign protein (antigen) may lead to the formation of two independent sets of antibodies, one responsible for the heightened resistance, and the other for the heightened vulnerability.

These earlier theories, however, did not find confirmation in subsequent investigations.

*From The Research Laboratories of the Western Pennsylvania Hospital, Pittsburgh, Pa.

Presented as a part of symposium on Anaphylaxis at the Second Pan-American Scientific Congress, Washington, D. C., Jan. 5, 1916.

At present the consensus of opinion among workers in this field of scientific endeavor, seems to point to the assumption that, in the measure in which the development of new properties in the blood of experimental animals is responsible for the establishment of either heightened or diminished specific resistance, the same set of substances is supposed to confer these outwardly contradictory changes in tolerance of animals to reinjection of foreign proteins.*

There remains, however, an open question as to the nature of these new properties of the blood serum, as well as to the mode of their action. Still less definite is the information as to the reason why these changes, brought about in experimental animals by the parenteral introduction of foreign protein, should produce a seemingly opposite effect on the natural tolerance of animals to these substances.

The changes in tolerance to a given protein, following a single or a multiple systematic introduction of the same protein into experimental animals, have been studied and variously interpreted by different investigators.

But for very slight minor differences, all the theories suggested can be brought back to two fundamental theoretical conceptions.

One, assuming after Ehrlich, that the foreign protein, introduced parenterally into the experimental animal, is anchored by such cells of the body, as happen to possess chemically active radicals (receptors), with specific chemical affinity for corresponding chemically active radicals of the protein introduced (antigen). The union of the respective radicals in virtue of their specific chemical affinity, is followed by the neutralization of the chemical avidity of the cell concerned, and by the subsequent restoration and overproduction by the cell, of the radicals thus saturated by antigen. This phenomenon (studied and described by Weigert), leads finally to the condition in which the cell finds itself overburdened with the radicals in question and at a certain time during the process, such a cell casts off into the circulation the excess of these chemically active radicals. Such substances, appearing in the circulation as a response of the cells to the introduction of a foreign body, are known under the name of antibody. The antibodies, according to this view retain, while free in the circulation, their power to combine with, and anchor the homologous protein, when such is reintroduced later.

Whereas the anchoring of antigen by the specific receptors of the cell is followed by incorporation of such antigen into the body of the cell, by virtue of the general digestive mechanisms of the cell, the detached receptors, circulating in the blood stream, combine with such specific antigen without digesting it, but merely changing such antigen in a manner in which it becomes more easily attackable by leucocytes, as well as by the complement, which is supposed to be the active principle directly responsible for "cytolytic" as well as "albuminolytic" properties of the blood serum.

According to the other fundamental theory, represented best by the work of Vaughan and his collaborators, the parenteral introduction of foreign protein stimulates the production of specific ferments within the body of experi-

*These views of course do not exclude the role body cells may have in the mechanism of specific protection.

mental animals. Such ferments, circulating in the blood stream, attack and digest directly the homologous protein when it is reintroduced later.

Although the above conceptions differ very substantially from each other on the question of the intimate nature of mechanisms governing the physiological processes, following the introduction of foreign protein into the animal body, both theories, in fact, indicate that such foreign protein undergoes parenteral assimilation, or digestion in the broader sense of the term, and thus eventually is eliminated from the circulation.

It is this element of ridding the body of the foreign material which is responsible for the view of the whole process as a protective mechanism, especially, because practically all the protein substances, when introduced parenterally, are apt to exhibit different degrees of toxicity, if the amount injected is sufficiently great.

Whichever of the two views upon the mechanisms of parenteral digestion is correct, the careful observers beginning with Jenner in 1798 and Magendi in 1839, have noticed repeatedly, that parallel with the apparent development of specific protective mechanisms, the parenteral introduction of protein foreign to the body, may give rise also to other mechanisms, detrimental to the well-being of the animal.

According to the views of the school following in the footsteps of Ehrlich, the reason for such paradoxic action of parenteral introduction of protein, is the fact that, during the extracellular specific lysis of formed, as well as unformed protein antigen in reinjected animals, this antigen is broken down, through the action of complement, and the poisons preexisting in the unaltered antigen are thus liberated.

Such a view has been especially plausible since Vaughan has shown that any protein can be broken up by chemical means, so as to yield a very powerful poison.

Friedberger, who deserves especial credit for most of the work in this direction, has shown that such poisons could be produced in the test tube by imitating the specific lysis, supposedly taking place in the body. In his earlier experiments Friedberger obtained from normal guinea-pig serum, which had been allowed to stand for some time with the washed specific precipitate (formed by rabbit serum immunized against sheep serum with the serum of the latter), very strong poisons which killed the guinea-pigs instantly with symptoms of acute shock. He named the poisonous substance anaphylatoxin, and assumed that it arose from the digestion of the specific precipitate, by the ferments of normal guinea-pig serum (complement).

According to the views of Vaughan and his followers, toxic phenomena in anaphylaxis are due to the split products of direct digestion of antigen, by the specific ferments present in the blood and tissues of sensitized animals.

More recently there has appeared a great number of publications concerning the question of the mechanism of the specific parenteral digestion. Abderhalden and his collaborators who are primarily responsible for this work, have taken up the theory of specific ferments and, by using specially devised methods, have seemingly demonstrated *in vitro* the presence of such ferments in the blood of prepared animals.

These findings, if correct, seem to offer a valuable link in the chain of reasoning of the earlier supporters of the theory of specific ferments.

Approaching the question of the mechanism of anaphylaxis at this stage of its development, the investigator is confronted with a fundamental question: Assuming that the anaphylactic shock is due to the liberation of poison in the body of sensitized animals, through the specific cleavage following the reinjection of antigen, what is the mechanism of this cleavage?

In order to answer this question, one must first determine the nature of changes taking place in the normal physiological processes of the animal, following the first experimental parenteral introduction of the foreign protein.

On the one hand, the hypothesis of Ehrlich suggests that such parenteral introduction of foreign protein is followed by the production of *specific antibodies.* On the other hand, Vaughan and his followers, without definitely denying the production of antibodies, assume that parenteral assimilation of foreign protein is followed by the output of specific ferments. In fact Abderhalden states definitely, that the parenteral introduction of foreign protein is followed by the production of specific ferments *parallel* with the production of specific antibodies, but independent of the latter.

If the interpretation of the phenomena observed by Abderhalden is correct, the production of specific ferments seems to be an even more general mechanism than that of production of antibodies. For antibodies have been thus far demonstrated only in cases of parenteral introduction of substances of animal or plant protein origin, provided that this protein is foreign to the species. Specific ferments, however, are claimed to have been demonstrated not only upon the parenteral introduction of such substances, but also upon that of proteins of homologous, and even autogenous nature, provided these substances are foreign to the blood ("blutfremd"). Moreover, the parenteral introduction of substances like gelatin, pepton, cane sugar, or casein, is claimed by the Abderhalden school to produce specific ferments, capable of attacking said substances, both *in vitro* and *in vivo.* Thus the group of substances which can play the part of antigen in the production of antibody, is included in that of the substances capable of causing the production of specific ferments, but is only part of it.

Such a conception of parenteral digestion, on first examination, seems very useful as a working hypothesis, for, if it is possible to prove the presence of such specific ferments, all findings from the realm of immunity can be brought into the sphere of biochemistry. The just criticism which the chemists had for a long time against the conception of immunologists, who dealt with "antibodies" and "alexins"—terms which seem to name, but not define, the still unknown substances,—would be immediately answered.

It is thus, that a number of men, familiar with phenomena of immunity, and anxious to find the general physiological principles underlying this very important mechanism, have turned their attention to the possibilities offered by the methods of investigation enunciated by Abderhalden.

In repeating the work of Abderhalden and his collaborators in this laboratory, however, we came to doubt their conclusions. Our experiments have shown that, although under certain conditions of experiment, the antigen may

be actually digested by the ferments of the blood of prepared animals, the ferments responsible for such digestion are not specific. In fact, as others have shown before us, the digestive ferments are present in every fresh serum, and if set free, can digest any suitably prepared substratum *in vitro,* without any specific predilection.

According to the findings of a number of investigators, the ferments normally present in the serum are usually inhibited by a simultaneous presence in the serum of antiferments. The removal of the latter liberates the active ferments and allows digestion to take place. Indeed Bordet, Nathan, Plaut, Flatow and others have demonstrated quite definitely, that mere mechanical adsorption by any of the substances like agar, kaolin, starch, and the like, may free serum of its antiferments and thus liberate the normal ferments.

On the other hand, as Schwartz, and more recently, Jobling and Peterson have shown, serum lipins may also inhibit the activity of normal ferments of the serum and their removal or (as we found it) even change in their solubility, may thus result in activation of the normal nonspecific ferments of the serum.

It is thus that a number of authors tried to explain the digestion, accredited by Abderhalden to the activity of newly produced specific ferments, by assuming that it was due to the mechanical adsorption of antiferments by the substratum and subsequent liberation of normal, nonspecific ferments.

Our own experiments, however, convinced us that such is not the case. We found that although the ferments concerned in the digestion are not specific (inasmuch as they can be made to digest any substratum), the phenomenon, as a whole, is not devoid of a certain degree of specificity and therefore could not be reduced to mechanical adsorption of antiferments.

Our systematic study of this question brought us to the conclusion that the element of specificity lies, not in the ferment itself, *but in the mechanism of its activation.* Namely, we found that the apparent specificity of ferment action *in vivo* assumed by Vaughan, and demonstrated *in vitro* by the methods of Abderhalden, is due to the fact that the combination of specific serum with its corresponding antigen, *in vivo* as well as *in vitro,* is followed by a radical change in the degree of dispersion of serum colloids. This physico-chemical change in turn is followed by the activation of a normal, nonspecific ferment of the serum.

If such a conception is correct, then the phenomenon of specific parenteral digestion may be explained on a basis very similar to that offered by the chemical theory of Vaughan, but instead of specific ferments, the digestion will be ascribed to normal nonspecific ferments, present in any fresh serum (complement?) and set free by a specific mechanism of combination between the antigen and antibody, very similar to that recorded by the meiostagmin reaction of Ascoli.

As was mentioned above, the toxic phenomena in anaphylaxis are ascribed by the chemical theory to the action of the split products of antigen, attacked by the active principle of the serum of the sensitized animal.

This hypothesis is based on observations, that cleavage of the antigen *in vitro* by chemical means yields poisonous split products; that combination of specific serum with its corresponding antigen yields toxic substances, identical

in their physiological action with those produced from the antigen chemically; and lastly the actual cleavage of antigen by the ferments of specific serum, can be demonstrated *in vitro*.

There exists, however, another view of the origin of so-called anaphylatoxin. This view represented by the work of Sachs, Nolf, Doerr, Ritz, Bordet, and others is known as "physical theory." In the main this theory suggests that the source of anaphylatoxin is not the antigen, but the protein of the serum itself.

As mentioned above, in his original experiment, Friedberger has succeeded in producing the toxic substances *in vitro* by a process similar to that which was supposed to be responsible for the anaphylactic shock *in vivo*. Namely, he allowed antigen to combine with its specific antibody and added the complement to this mixture. The poisons obtained in this manner *in vitro*, when injected into normal animals, were able to produce typical anaphylactic symptoms.

In his later experiments, however, Friedberger succeeded in obtaining similar poisons from bacteria and other proteins by their incubation with normal guinea-pig complement without the concurrence of specific antibody. He still believed, however, that in these experiments also the poison was derived from the protein of the substratum. Even when Bordet, Nathan, Mutermilch and others have been able to obtain similar poisons by the incubation of normal guinea-pig serum with agar, starch, or kaolin, and have decided therefore, that the poison must originate from the serum, Friedberger still objected to these conclusions on the basis that these substances may contain a small amount of protein impurities, which really furnish the substratum for the formation of anaphylatoxin.

In studying this controversy one finds two really independent questions involved in it. The first question seems to be: "What are we to take to be the anaphylatoxin?" It is only after agreeing as to the exact meaning of this term, that one can attack the main question: "Which is the source of poisonous substances in the anaphylatoxin formation—is it antigen or the serum?"

Originally, the term "Anaphylatoxin" was applied by Friedberger to designate the poison which was supposed to be identical with the one produced *in vivo* during the anaphylactic shock. This poison had two essential characteristics, making it possible to assume its identity with the substance causing anaphylaxis. First, the method of its production from combination of antigen with antibody and complement, and second, its physiological action upon normal animals, which is identical with anaphylactic shock.

In discussing the question of the origin of anaphylatoxin, different authors apparently used as criterion in their terminology only the second characteristic of the original anaphylatoxin—namely, its physiological effect upon normal animals. Thus, many authors have called anaphylatoxin the chemical poisons obtained by Vaughan from bacteria; Nathan, Bordet and Mutermilch called anaphylatoxins substances obtained by them from the serum by adsorption with inert substances, etc. Attempts of these authors to explain the nature of anaphylatoxin seem to be generally inadequate, because, even though, as my experiments have also confirmed, by digestion of serum with kaolin, for instance,

one can produce from serum a substance which is similar in its physiological action, on the one hand, to the original anaphylatoxin of Friedberger, and, on the other hand, to the chemical poison of Vaughan, its identity with one or the other is not proved by this similarity alone. The identity between all these substances has to be proven before the experiments above could be taken into consideration in discussing the probable nature of anaphylatoxin.

This we attempted to do in a series of experiments in which we studied the actual mechanism of the production of poison in each instance. These experiments have shown that in the poisons arising during the incubation of the serum with kaolin or starch, as well as those arising when specific serum is digested with its antigen, the serum is the source of poisonous products. Moreover, it was found that the products of such autodigestion of serum are toxic only to homologous animals. This would indicate that during the anaphylaxis *in vivo* the toxic split products originate only from the digestion of serum or tissues of the animal and not from heterologous protein of the antigen.

Since it is established that in the experiments of Bordet, as well as in those of Friedberger, the formation of toxic split products is identical, namely, that it is due to the digestion by the normal serum ferments of autogenous protein, the similarity of the biologic properties of the respective end-products of such digestion may speak for their identity. On the other hand, the fact that in Vaughan's experiments the poison arises from heterologous protein shows that this poison is not identical with anaphylatoxin, in spite of the similarity of its biologic action upon animals.

These findings, taken in connection with the results obtained by us in the study of the mechanism of specific parenteral digestion, referred to above, suggest that the nature of anaphylatoxin is as follows: Fresh serum contains normal proteolytic ferments whose digestive action *in vivo* as well as *in vitro* is inhibited by the simultaneous presence of some antitryptic elements. This antitrypsin can be removed from the serum *in vitro* by two independent processes: one, nonspecific,—a simple mechanical adsorption by means of excess of some organic as well as some inorganic substances; the other, specific,—an inactivation of the antitryptic properties of the serum, taking place as a result of the physico-chemical changes in the serum, induced by the specific interaction between the antigen and the antibody of the immune serum. The removal of the inhibiting antitryptic action of the serum, by either method, is followed by the restitution of the activity of the normal proteolytic enzyme, which may attack both the protein of the antigen, as well as the protein of the serum itself. At a certain stage of this autodigestion the split products of the serum protein exhibit toxic properties. Biological properties of these toxic substances indicate their similarity to the anaphylatoxin and suggest that the anaphylatoxin of Friedberger, whether occurring *in vivo,* or produced *in vitro,* is a result of the autodigestion of serum, and not of the protein outside of the serum.

Our findings as described above showed that there is no experimental evidence of the existence of specific ferments, and that the phenomena of parenteral digestion, ascribed by Vaughan to the activity of specific ferments, can be plausibly explained and experimentally demonstrated, without assuming the existence in the body of a special specific mechanism outside of antibody formation.

Having thus answered the first question which we set out to study, we will try to answer the second one.

Namely,—assuming the above mechanism of parenteral digestion to be correct—why then in some cases, does the introduction of protein into normal animals result in the establishment of heightened resistance, whereas in other cases it leads to heightened vulnerability?

As we have stated above, the activation of normal ferments present in specific sera, through the changes in colloidal dispersion following the union *in vivo* between the antigen and antibody, may lead to digestion of antigen as well as to that of the serum itself. When the antigen mainly is digested, the phenomenon is interpreted by the observer as that of protection. When the serum or other autogenous elements are digested, the autogenous split products being toxic to the animal, the intoxication occurs and the observer interprets symptoms as a sign of heightened vulnerability.

It is evident from the above, that the actual mechanism of parenteral digestion in the body of the animal previously injected with the homologous protein, and thus possessing the circulating specific antibody, is the same in both cases. Namely,—the antigen upon its reintroduction is anchored by antibody, and this union leads to the liberation of normal proteolytic ferments of the serum. It is only at this point that the difference may come in, depending upon which substratum is mainly attacked by this ferment.

There must be at this point, therefore, the main problem of the situation. What determines the direction of the activity of the ferments? Is this activity selective and directed exclusively toward sensitized antigen in one case, and the serum protein in the other, or is the difference in two cases only quantitative and not qualitative?

Our experiments point to the second, as a correct answer.

It is true, some of our findings seem to show that sensitized antigen is more readily digested by the ferment in *"momentu nascendi"* and thus there might be a degree of selective action on the part of a ferment. But experiments in this direction are very difficult and the amount of work actually done is not yet sufficient for us to definitely claim such to be the case.

On the other hand, and in the majority of experiments, there seems to be no selective action on the part of the ferment. Both the serum itself and the antigen seem to be equally subject to the attack.

What then determines the degree of toxicity developed during this process of parenteral digestion?

First of all, of course, the amount of the autogenous toxic split products liberated during digestion. And this seems to be in direct relation with the amount of antigen introduced.

It is very well established experimentally that, no matter how small it may be in certain cases, the dose of antigen introduced into a sensitized animal must be sufficient to produce the anaphylactic shock. If the amount of antigen is too small, the shock does not take place, although the experimental animal may show other, milder symptoms, due to partial intoxication.

It is thus evident, that the amount of antigen reintroduced into a sensitized animal, is a very important factor in determining the degree of intoxication.

On the other hand experiments have definitely shown, that exposure of sensitized animals to cold or starvation may reduce the toxic effect of the injection of a lethal dose of antigen.

These experiments suggest, that not only the actual amount of antigen introduced, but also the rapidity with which it unites with the antibody, may influence the rapidity of liberation of ferments and resulting intoxication.

This relation may be still better demonstrated by the known fact, that if instead of injecting at once a lethal dose of antigen into a highly sensitized animal, one would inject the same and even a larger amount very slowly, the acute anaphylactic shock might be averted. Thus, the degree of toxicity developed during parenteral digestion in sensitized animals is apparently determined by the amount of digestion taking place in a unit of time and this in turn depends on the amount of antigen injected in a unit of time.

Such a view of the phenomenon would explain, it seems, how it is that parenteral digestion, following reintroduction of antigen, may at times seemingly protect the animal, whereas at other times injures its well-being.

If the amount of antigen reintroduced into a sensitized animal is small or introduced very slowly, the amount of ferment liberated in unit of time, is very slight, and the amount of autogenous split products may be so small, that the animal may show no apparent symptoms of intoxication. If, however, the amount of antigen is sufficient and if it is introduced rapidly, the rate of activation of the ferment is great, and the animal succumbs to intoxication with autogenous split products.

In case of natural reinfection with pathogenic microorganisms, the amount of antigen, which penetrates into the body of sensitized animals, is usually small enough, and even if it multiplies in the body at the beginning, the process is so slow and the extent of autodigestion in a unit of time is so slight, that the reaction occurs without the visible injury to the host, and this is why the observers called the process *"protective"* and the animal *"immune."*

If, however, the same animal be artificially given a large dose of the same antigen, large amount of ferment is liberated rapidly, causing anaphylaxis and we call the same animal *"hypersensitive."*

However, the amount of the antigen and the rate of its introduction into sensitized animals is apparently not the only mechanism controlling the rate of parenteral digestion, as judged by the appearance of toxic split products.

It is observed for instance, that animals, receiving systematic injections of toxic foreign protein *at short intervals,* can develop their resistance to the given toxic substance to such a degree that the amount of protein containing several toxic doses of antigen, may be injected, no matter how rapidly, without producing even the slightest symptoms. It is noticed, however, that a similar animan inoculated with a similar dose of antigen, the only difference being that between the time of the last serial injection and the test injection sufficient time has elapsed—will succumb with anaphylactic shock.

In both cases the amount of antigen and the rapidity of injection being the same, it is evident that the difference in time elapsed between the last serial

injection and the test injection, determines the difference in the effect of the respective test injections.

Just what happens in the sensitized animal during this period of time, called "incubation period," is differently explained by different authors. Our own experiments lead us to believe that the reason for the difference in the response of the sensitized animals to reinjection in the two cases cited above, is as follows:

The union between the antigen and antibody, as we have suggested above, reduces the inhibiting power of the serum-antitrypsin, and thus liberates its normal ferments. Such can be the case only as long as the remaining amount of antiferment is not too great to interfere with the action of the ferment, as it is liberated. If the amount of antiferment is too great, it may delay, or altogether prevent, the digestive action of the ferments.

When antigen is introduced parenterally into a normal animal, once or in serial injections, such antigen is digested. It is known that the products of such digestion are strongly antitryptic. It is thus, that as the digestion progresses, there is going on all the time the formation of new antitryptic split products, which are eventually either assimilated by the cells in the constructive process of the body, or are eliminated. Such removal of excess of antitryptic elements is, however, not rapid, and as long as the antitryptic split products of the antigen from the previous injection remain in circulation, the rapid action of newly liberated ferments is prevented, and anaphylaxis does not occur. If, however, enough time is allowed to pass, usually about twelve to fifteen days, before the test injection is given, the excess of antitryptic products of previous parenteral digestion are removed, and a rapid action of ferments is thus made possible. Such rapid digestion may cause the shock.

That this view is correct also follows from the analysis of the phenomenon of so-called *vaccination* against anaphylaxis.

It was my good fortune to study the phenomenon of anaphylaxis in Besredka's laboratory when he found that, if the treated animal should be given a second injection of antigen before the expiration of full incubation time after the first injection, such an animal does not respond with anaphylactic shock to the test injection at the time expected (thirteen to fifteen days after the first injection), but later. Moreover, the larger the dose of antigen injected, the longer is the state of hypersensitiveness delayed, and the period of resistance prolonged.

This phenomenon suggested to Besredka the possibility of using it, in order to prevent the undesirable reaction in anaphylactic animals.

Namely, he succeeded in preventing anaphylactic shock in animals having passed the incubation period after their first (or after their last serial) injection, by merely injecting a sublethal dose of the antigen some short time previous to the following test injection of several lethal doses of antigen.

This experiment is the basis of the method, which is used every day now for the prevention of serum sickness in children receiving more than one injection of diphtheria antitoxin.

The mechanism of such *"vaccination against anaphylaxis"* was found by us to be that of retardation of ferment action by the circulating split products

remaining from the digestion following the vaccinating injection of antigen, and not to exhaustion of antibody, as assumed by others.

Such a state of seeming resistance to anaphylaxis, or as it is called *"the state of antianaphylaxis,"* was produced by many other procedures than vaccinating injection of antigen.

Thus, it was found that administration of anesthetics, sedatives and many other toxic substances may prevent the subsequent development of anaphylaxis. The mechanism of such action of the substances just referred to, was never adequately explained.

Having convinced ourselves that in the case of *"vaccination"* against anaphylaxis the mechanism involved was that of production of antitrypsin, we tried to see if the same could also be true in the other cases. Actual measurements have shown this to be the case. We found that without exception, the administration of substances known to have the effect of preventing the anaphylactic shock, is followed by a more or less marked increase in antitryptic properties of the blood.

Thus we suggest that administration of poisons, causing destructive changes in the cells of the body, in quantities not sufficient to kill the animal outright, is followed by the death of the tissues immediately affected by the poison. With the death of tissues the intracellular ferments are set free. These ferments, possibly with the collaboration of the ferments thrown out from the surrounding fixed cells, as well as from the blood serum and leucocytes, proceed to dispose of the dead material.

Some of the protein split products of such digestion, together with some of the nonprotein constituents of the destroyed cells, may exert antitryptic action. If a sensitized animal is subjected to such treatment, previous to the test injection, and if such test injection is given before the antitryptic split products referred to above are eliminated, they may retard or stop the activity of proteolytic ferments liberated upon the introduction of antigen, and thus prevent the anaphylactic shock.

The view of specific parenteral digestion, as outlined in the earlier part of this paper offers, it would seem, a plausible basis on which the various seemingly contradictory phenomena of immunity can be satisfactorily explained. Such a hypothesis suggests first of all, that the terminology used in connection with the study of the reactions of the living organism, following its invasion by biological poisons, is not adequate.

In considering the question in the light of its historical development, one is impressed by the fact that ever since the first observations on this subject, the apparent increase of tolerance to biological poisons, following their repeated introduction into experimental animals, has been accepted to be the expression of nature's protective force. This *teleological* conception of the reaction on the part of animal organisms upon the parenteral introduction of foreign material, however, could not adequately explain all the phenomena observed. The work of Vaughan especially has suggested that in spite of the difference in the final effect, the reaction of the animal body must be the same in case of heightened as well as diminished resistance to poisons.

Although our experiments fully confirmed this view, the intimate mechanism of this reaction seems to be different from that suggested by Vaughan.

There seems to be no evidence of the existence of specific ferments and the apparent specificity of digestive processes seems to be due to the presence in sensitized animals of specific antibodies in the sense of Ehrlich.

As to the difference in final effect following the specific activation of serum ferments—it is due to the difference in rate of digestion.

Thus phenomena "immunity" or "anaphylaxis" can be nothing but different forms, in which a greater and more general process of specific parenteral digestion expresses itself to the observer.

THE SEROTOXIN OF JOBLING*

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THE production of a serotoxin by treating homologous and heterologous sera with chloroform and ether as reported by Jobling[1] has found wide acceptance among workers in the field of anaphylatoxins and is incorporated in the literature of the subject alongside the earlier pioneer work of Richet, Bordet, Friedberger, Nathan and others. Jobling maintains that the ferment action of the serum is held in abeyance normally by an unsaturated lipoidal antitryptic substance; the removal of which by lipoid solvents permits autolysis of the serum proteins, thereby forming a poison, which is in all probability identical with Vaughan's protein split product.

Some work was under way in this laboratory in which splitting of germ substance by sera was the desired end. In the light of Jobling's work it seemed reasonable to assume that the splitting action of the sera could be greatly increased and accelerated by shaking out the sera with chloroform before incubation with the germ substance. Therefore rabbit serum was shaken with one-tenth its volume of chloroform four minutes, then centrifuged at 8,000 R.P.M. for ten minutes and the supernatant serum carefully pipetted off from a precipitate that, upon centrifugation, was interposed between the chloroform on the bottom and the serum above. More will be said later concerning this precipitate. As a matter of routine, controls were made by testing the action of the serum on guinea-pigs before incubation with the germ substance. As high as 9 c.c. of the normal untreated serum had been injected without effect, but since the M.L.D. of serum and germ substance after incubation was known by trial to be 3 c.c. or less, the control injection of the normal serum was usually limited to 3 c.c. But upon the injection of the chloroformed serum in 3 c.c. quantities as a control; that is, without incubation with germ substance, the pigs died instantly. Reduction of the dose to one cubic centimeter still produced death in most cases, and always a marked prostration. A summary of these observations is given in table I.

*From the Hygienic Laboratory, University of Michigan.

TABLE I.

Rabbit serum shaken at room temperature with one-tenth volume of chloroform, centrifuged ten minutes at 8,000 R.P.M.

PIG.	DOSE.	RESULT.
1	3 c.c.	Instantaneous death.
2	2.5 c.c.	" "
3	2 c.c.	" "
4	1.5 c.c.	Prostration; died in four hours.
5	1 c.c.	Instantaneous death.
6	1 c.c.	Deep prostration; lived.
*7	3 c.c.	No effect.
*8	3 c.c.	No effect.

*Controls; received normal untreated serum.

The character of the deaths, together with the autopsy findings clearly indicated atypical anaphylaxis and pointed strongly to the *residual chloroform* in the serum as the toxic agent. The serum was perfectly clear and free from emulsion by reason of the thorough centrifugation, but had a faint odor of chloroform. To determine roughly the influence of the residual chloroform, a portion of the serum was filtered six times through qualitative paper and 3 c.c. then injected, intravenously as before. The pig was beautifully anesthetized for a few minutes, but recovered sharply and completely, in a manner very similar to the recovery of those pigs receiving a sublethal dose of the treated serum without filtration. Another portion of the treated and centrifuged serum was placed in a wide mouth test tube and air blown over the surface for two hours. Bubbling air through the serum was impracticable because of the foaming. Injection in 3 c.c. doses produced death, but only varying degrees of stupor in lesser quantities, 1 c.c. being entirely without effect. Again, some normal serum was shaken with one per cent its volume of chloroform for exactly one minute, then allowed to subside for five minutes and the serum carefully pipetted off. Three cubic centimeters of this serum produced an immediate and deep anesthesia lasting for five minutes, when the pig recovered as sharply as it had been prostrated.

All this is preliminary to the object of this paper and only relates the manner in which our attention was temporarily diverted from another line of work to a consideration of certain very obvious phases of Jobling's serotoxin studies. Several objections can be raised at this point to any assumptions, on the basis of the experiments thus far cited, concerning serotoxin. First, rabbit serum is at best but weakly tryptic, and would therefore be unlikely to produce a serotoxin of any remarkable potency. Again, the quantity of chloroform used was too small, according to Jobling, to produce a serotoxin by lipoid removal. Lastly sufficient time was not allowed for the tryptic action to assert itself in full force.

Accordingly various sera were tried and the method of procedure modified to give better conditions for the serotoxin to be formed. In the next experiment rabbit serum was sealed in tubes with chloroform in the proportions of one-tenth volume, one volume, and two volumes of the chloroform to that of the serum. As control tubes, physiological salt solution with the same proportions of chloroform were made up and a tube of the serum with no chloroform. All were placed on the shaking machine at room temperature, and agitated for

the times indicated in the tabulations below, then centrifuged at 8,000 R.P.M. for thirty-five to sixty minutes, and further treated as specified in each protocol.

TABLE II.

Rabbit serum plus one-tenth volume of chloroform, shaken 36 hours at room temperature, centrifuged 35 minutes at 8,000 R.P.M.

PIG.	DOSE.	RESULT.
1	1.5 c.c.	Death in 6 minutes; no shock, some dyspnea, lungs dilated and clot in the heart.
1	1 c.c.	Death in 2 minutes; slight dyspnea, no clot, no dilation of the lungs.
3	1 c.c.	Prostration; recovery sharp and complete.
4	.5 c.c.	No effect.
		A portion of the treated and centrifuged serum was aerated for 15 minutes by blowing air through it from a very fine capillary.
5	1 c.c.	No effect.
6	1.5 c.c.	Slight prostration; lived.
7	2 c.c.	Prostration 2 minutes; lived.
8	3 c.c.	Prostration 3 minutes; lived.
*9	1 c.c.	Slight shock; lived.
*10	1.5 c.c.	Marked shock; lived.
*11	3 c.c.	Typical anaphylactic death and autopsy.

*Controls; received serum shaken 36 hours with no chloroform.

TABLE III.

Rabbit serum shaken 36 hours with one volume of chloroform, then centrifuged 35 minutes at 8,000 R.P.M.

PIG.	DOSE.	RESULT.
1	1 c.c.	Prostration; lived.
2	2 c.c.	Deep prostration; recovery gradual and slow.
3	2 c.c.	Death in 2 minutes; atypical, autopsy likewise.
		A portion was aerated 45 minutes by blowing air through a fine capillary.
4	2 c.c.	No effect.
*5	2 c.c.	Severe prostration; recovered.
*6	3 c.c.	Death in 2 minutes; atypical death and autopsy.

*Controls; received physiological salt solution treated with two volumes of chloroform, shaken 36 hours, centrifuged 35 minutes at high speed.

In the next experiments two radical modifications were made in the procedure. The centrifugation is continued at the usual high rate for one hour. The aeration was accomplished by placing the serum after treatment and centrifugation, in a wide tube and then directing a strong current of air tangentially against the surface of the serum close to the wall of the tube. The serum by this method was set into violent agitation, but the troublesome foaming was entirely eliminated. The air used was filtered through a long tube filled with glass wool.

TABLE IV.

Rabbit serum plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

PIG.	DOSE.	RESULT.
1	1 c.c.	Stupor; lived.
2	2 c.c.	Deep prostration; recovered.
3	2 c.c.	Instantaneous death; atypical symptoms and autopsy.
4	2.5 c.c.	Death in 1 minute; atypical, autopsy likewise.
		A portion was aerated 30 minutes by surface agitation.
5	5 c.c.	No effect.
*6	3 c.c.	No effect.
*7	5 c.c.	No effect.

*Controls; received serum when fresh and without treatment.

TABLE V.

Rabbit serum plus one volume of chloroform, shaken 18 hours, centrifuged one hour.

PIG.	DOSE.	RESULT.
1	1 c.c.	Prostration 2 minutes; recovery sharp and complete.
2	2 c.c.	Death in 2 minutes; atypical.
	A portion was aerated 30 minutes by agitation.	
3	2 c.c.	No effect.
4	2 c.c.	No effect.
	Another portion was aerated 45 minutes by agitation.	
5	4 c.c.	No effect.
6	5 c.c.	No effect.
*7	3 c.c.	Death in 5 minutes; atypical.
*8	3 c.c.	No effect.
*9	3 c.c.	Slight intoxication; no shock.
#10	5 c.c.	No effect.
#11	5 c.c.	No effect.

*Controls; received serum shaken 18 hours without chloroform.
#Controls; received normal serum standing in cold 18 hours.

TABLE VI.

Physiological salt solution plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

PIG.	DOSE.	RESULT.
1	2 c.c.	Deep prostration; lived.
	Aerated 30 minutes by bubbling air through the solution.	
2	5 c.c.	No effect.
*3	5 c.c.	No effect.

*Control; received physiological salt solution.

TABLE VII.

A .5% solution of chloroform in physiological salt solution was shaken 18 hours. Centrifugation was omitted as no chloroform settled out upon standing.

PIG.	DOSE.	RESULT.
1	2 c.c.	Instantaneous death.
	Solution was diluted to two volumes with salt solution.	
2	2 c.c.	Prostration and slow death in 5 minutes.
3	1 c.c.	Prostration 1 minute; lived.

We next wished to show that the clarity of the serum was no proof that it was free from chloroform. Rabbit serum was placed with two volumes of chloroform, with occasional shaking at room temperature four days. It was then centrifuged for exactly ten minutes, which was not sufficient centrifugation to make a complete separation of the emulsion, as was evidenced by the cloudiness of the serum. A series of filtrations was then begun with injections intervening to check the progressive partial removal of the residual chloroform. The results are shown in table VIII.

TABLE VIII.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	NO. OF FILTRATIONS.	RESULT.
1	350	.5 c.c.	.0014	0	Prostrated 6 minutes; lived.
2	290	.75 c.c.	.0026	2	No effect.
3	220	1.5 c.c.	.0068	4	Atypical death in 19 minutes.
4	275	2.0 c.c.	.0072	7	Prostrated 3 minutes; lived.
	An unfiltered portion was aerated 20 minutes by agitation.				
5	225	3.0 c.c.	.013	0	Mild shock; lived.
*6		3.0 c.c.			No effect.
*7		5.0 c.c.			No effect.

*Controls; received fresh untreated serum.

After the fourth filtration some of the filtered serum, which was now crystal clear, was placed in a side arm test tube, tightly corked and the side arm connected to a suction pump giving 24 inches of vacuum. The tube with contents was placed in a bath at forty-five degrees C. for 30 minutes, the pump working all the time. Violent ebullition of the serum began almost immediately and lasted about four minutes. Table IX indicates the results of the injections.

TABLE IX.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	215	1 c.c.	.0046	No effect.
2	225	3 c.c.	.013	Shock; lived.
*3	235	3 c.c.	.0127	Light shock; lived.

*Control; received serum which had stood at room temperature 4 days.

At the same time that the serum used in tabulations VIII and IX was placed with the chloroform, another portion was placed with two volumes of ether and with occasional shaking was allowed to stand at room temperature seven days. It was likewise centrifuged ten minutes and the serum removed from underneath the ether by means of a bulb pipette provided with a very fine capillary stem, so as to minimize the quantity of ether which would unavoidably be taken in as the tube penetrated the ether to reach the serum. Table X records the injections and results.

*TABLE X.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	245	1 c.c.	.0041	Slight prostration.
2	275	1 c.c.	.0036	Prostration 3 minutes; lived.
3	245	1.5 c.c.	.0061	Prostration four minutes; lived.
	The remainder was aerated 30 minutes by agitation.			
4	250	2 c.c.	.008	No effect.
5	285	3 c.c.	.011	No effect.
6	225	4 c.c.	.018	No effect.

*Controls same as in table eight.

In table XI is condensed the last experiment on rabbit serum. This was done in order to make a comparison of the two methods of chloroform removal on the same treated serum.

TABLE XI.

Rabbit serum plus one volume chloroform, shaken 22 hours, centrifuged one hour.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	340	.5 c.c.	.0015	Slight stupor.
2	400	1 c.c.	.0025	Death in 4 minutes; atypical autopsy.
	A portion was aerated 30 minutes by agitation.			
3	400	2 c.c.	.005	Light shock; lived.
4	285	3 c.c.	.011	No effect.
	A portion was placed at 45 degrees C. under a 24 inch vacuum for 5 minutes. Serum boiled vigorously for 2-3 minutes.			
5	295	1 c.c.	.0034	No effect.
6	270	2 c.c.	.0074	Prostration 4 minutes; lived.

Still another portion was left at 45 degrees C. for 30 minutes, the vacuum pump acting continuously.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
7	285	3 c.c.	.011	Light shock; lived.
*8	335	3 c.c.	.009	No effect.
*9	315	5 c.c.	.016	No effect.
#10	290	3 c.c.	.01	No effect.
#11	240	5 c.c.	.021	No effect.

*Controls; received fresh normal serum.
#Controls; received serum shaken 22 hours without chloroform.

The same procedure as has been given thus far was followed quite closely in the work on other sera than rabbit. The data will be tabulated for each serum, and such minor modifications as were made noted.

TABLE XII.

Dog serum plus two volumes of chloroform, shaken 18 hours, centrifuged one hour.

PIG.	DOSE.	RESULT.
1	1 c.c.	Instantaneous atypical death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Shocked; died in 2 hours, typical autopsy.
3	2.5 c.c.	Moderate shock; lived.
4	1 c.c.	Mild shock; died in 12 hours.
*5	3 c.c.	Death in 7 minutes; typical autopsy.
*6	2 c.c.	No shock; died in 15 hours.
*7	2.5 c.c.	Typical shock and death in 6 minutes; autopsy likewise.
*8	2 c.c.	Death in 1½ hours.

*Controls; received fresh normal dog serum.

TABLE XIII.

Dog serum plus one volume of chloroform, shaken 18 hours, centrifuged 1 hour.

PIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Violent shock and death in 23 minutes; typical autopsy.
3	2 c.c.	Shock and death in 31 minutes; lungs contracted, other autopsy findings typical.
4	1 c.c.	Mild shock and death in 15 minutes; typical autopsy.
5	2.5 c.c.	Violent immediate shock followed by gradual collapse and death in 43 minutes; no clot, lungs contracted.
*6	2.5 c.c.	Death in 18 hours.
*7	3 c.c.	Death in 2 hours; no shock, typical autopsy.
*8	3.5 c.c.	Typical death in 7 minutes; autopsy likewise.

*Controls; received dog serum shaken 18 hours without chloroform.

TABLE XIV.

Dog serum plus one-tenth volume chloroform, shaken 18 hours, centrifuged one hour.

PIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
Aerated 30 minutes by agitation.		
2	2 c.c.	Immediate shock; died in two days.
3	1 c.c.	Light shock; lived.
4	2.5 c.c.	Moderate shock; lived.
5	3 c.c.	Shock; lived.
*6	2.5 c.c.	Shock; lived.
*7	3 c.c.	Shock; and typical death in 39 minutes, autopsy likewise.
*8	3.5 c.c.	Typical death in 5 minutes.

*Controls; received normal dog serum standing in cold for 18 hours.

TABLE XV.

Dog serum plus one volume of chloroform, shaken 19 hours, centrifuged one hour.

PIG.	WT.	DOSE.	C.C. PER GM. OF WT.	RESULTS.
1	400	1 c.c.	.0025	Instantaneous death.
2	280	.5 c.c.	.0018	Death in 30 seconds.
	Aerated 45 minutes by agitation.			
3	260	3 c.c.	.012	Death in 30 minutes; lungs contracted, no clot, heart in diastole and beating.
4	230	2 c.c.	.0087	Mild immediate shock; died in 14 hours, autopsy same as #3.
5	260	3 c.c.	.012	Death in 25 minutes; autopsy same as #3.
6	175	1 c.c.	.0057	Death in 2½ hours; no shock, autopsy same as #3.
7	270	3.5 c.c.	.013	Death in two hours; autopsy same as #3.
*8	300	3 c.c.	.01	Immediate shock; death in 14 hours.
*9	350	3.5 c.c.	.01	Immediate shock; death in 3 hours.
*10	250	3 c.c.	.012	Shock and death in 6 minutes; autopsy same as #3.
*11	240	3 c.c.	.0125	Immediate shock and death in 23 minutes; autopsy same as #3.
*12	400	5 c.c.	.0125	Immediate shock; death in 2½ hours, autopsy same as #3.
*13	260	3 c.c.	.0115	Immediate shock and death in 7 minutes; autopsy same as #3.
!14	240	3 c.c.	.0125	No effect.
!15	225	3.5 c.c.	.015	No effect.
!16	240	4 c.c.	.017	Immediate shock; death 1½ hours, autopsy typical.
!17	190	3.5 c.c.	.018	No effect.

*Controls; received fresh normal dog serum.
!Controls; received dog serum shaken 19 hours without chloroform.

TABLE XVI.

Guinea-pig serum plus one volume of chloroform, shaken 21 hours, centrifuged one hour.

PIG.	DOSE.	RESULTS.
1	.5 c.c.	One minute prostration.
2	1 c.c.	Deep prostration.
	Aerated 30 minutes by agitation.	
3	4 c.c.	No effect.
4	5 c.c.	No effect.
*5	3 c.c.	No effect.
*6	3.5 c.c.	No effect.
#7	5 c.c.	No effect.

*Controls; received homologous serum shaken 21 hours.
#Controls; received homologous serum after standing in cold 21 hours.

TABLE XVII.

Rat serum plus one volume chloroform, shaken 17 hours, centrifuged one hour.

PIG.	DOSE.	RESULTS.
1	1 c.c.	Atypical death in one minute.
	Aerated 45 minutes by agitation.	
2	2 c.c.	Shock; lived.
3	3 c.c.	Typical death in 5 minutes.
4	2 c.c.	Shock; lived.
5	2 c.c.	Mild shock; lived.
6	2.5 c.c.	Typical death in 3 minutes.
7	1 c.c.	Severe shock; lived.
*8	3 c.c.	Typical death in 2 minutes.
*9	2 c.c.	Mild shock; lived.
*10	3 c.c.	No effect.
#11	3 c.c.	Typical death in 2 minutes.

*Controls; received fresh pooled rat serum.
#Controls; received normal rat serum shaken 19 hours without chloroform, becoming slightly coagulated.

TABLE XVIII.

Cat serum plus one volume of chloroform, shaken 22 hours, centrifuged one hour.

PIG.	DOSE.	RESULTS.
1	1 c.c.	Instantaneous death.
	Aerated 30 minutes by agitation.	
2	2 c.c.	Slight shock followed by slow collapse and death in one hour.
	Further aerated for 15 minutes by agitation.	
3	2 c.c.	No effect.
4	3 c.c.	No effect.
5	4 c.c.	No effect.
*6	3 c.c.	No effect.
*7	5 c.c.	No effect.
#8	4 c.c.	No effect.
?9	4 c.c.	No effect.

*Controls; received fresh normal cat serum.
#Controls; received serum shaken 22 hours without chloroform.
?Controls; received normal serum after standing in cold 22 hours.

DISCUSSION.

A study of the data given in the preceding tables shows the sera to fall sharply into two groups on the basis of their toxicity either before or after treatment with the chloroform. The first group is composed of the sera of the rabbit, cat, and the homologous serum, guinea-pig. All these when fresh are normally atoxic, and fail after treatment to show the production of anaphylatoxin. The other group is that of rat and dog sera. In this case the question becomes one of the increase of the toxicity by the chloroform treatment, since both are variably normally toxic for guinea-pigs. In table XII, animal 7 shows a typical death and autopsy with an injection of 2.5 c.c. of fresh dog serum. Animal 6 of the same table died in 15 hours, receiving only 2 c.c. of the same serum, but the length of time involved should rule out this and all other deaths occurring more than 30 minutes after the injection. In table XIII, animal 4 showed a typical death in 15 minutes, receiving but one cubic centimeter of the treated serum. The M.L.D. of the untreated serum is observed to be 3 c.c.

With rat serum the M.L.D. was likewise 3 c.c. for the normal serum; after treatment it dropped to 2.5 c.c. Injection of other quantities of both treated and untreated rat and dog sera, produced shocks, but such effects are rather characteristic of both these sera, their toxicity seeming to depend upon some labile conditions that are far from being constant in their action upon injection. Between these extremes there is observed to be considerable common ground to the treated and the untreated rat and dog sera, in which the toxic effects are nearly parallel.

In table VII is shown how small a quantity of chloroform is required to produce death or marked prostration effects when intravenously given to guinea-pigs. This point was tested again and again and the data given is only representative of a large number of experiments made to determine rather closely the M.L.D. of a solution of chloroform. From sera, chloroform is removed only with great difficulty, and since they do not retain chloroform to an equal degree one is never sure when it is completely removed except by trial upon animals. In our work 30 to 45 minutes aeration was found to be required. The dog serum always came from the centrifugation foggy in appearance. In this case the emulsion formed was so fine that centrifugation at 8,000 R.P.M. for

one hour failed to completely remove it. Upon agitation with air for 5 to 10 minutes, or filtration through qualitative paper 4 to 6 times, the serum would become crystal clear, which might easily be assumed to indicate a complete removal of the chloroform. Injection tests running alongside such repeated filtrations, as recorded in table VIII, show clearly that filtration will not remove the chloroform sufficiently, but that it will give marked effects upon injection. Further by applying a very delicate test for chloroform we were able to show that even after the supposedly complete removal by any of the rather strenuous methods employed, there still remained easily detected quantities of chloroform. The method of detection was to make use of the carbylamine reaction,[2] represented by the equation;

$$C_2H_5NH_2 + CHCl_3 + 3KOH = 3KCl + 3H2O + C_2H_5NC.$$

About equal quantities of the serum and alcoholic potash[3] are placed in a tube with one drop of aniline. Upon heating the characteristic odor of carbylamine is evolved. One drop of the treated serum before chloroform would give a most pronounced test, and the test never failed when applied to the sera after removal of the chloroform by any of the drastic means employed. How great the influence of this minute quantity of chloroform might be is only a matter for conjecture, however it would seem logical, that since 2 c.c. of a .25 per cent salt solution of chloroform will kill, that much smaller quantities accompanying the serum would not be entirely without effect.

The wide divergency in the character of the deaths of the animals dying from the treated serum as compared to those dying from the untreated serum was most striking. Those animals receiving the first injection of the serum after centrifugation, before any chloroform removal had been attempted, would in nearly every case be dead before the injection could be completed. When the chloroform was but partially removed, a sudden and very typical anesthesia, designated in the tables as prostration, would ensue. The normally toxic sera would give the characteristic jerks of anaphylaxis when untreated, and the same thing would occur after treatment, except that the animal would always be prostrated, making no attempt to gain its feet or to move about. In some instances such animals would never revive, but after a shock of varying intensity, would lie for hours immovable, and the exact time of their death would be uncertain as the typical dyspnea of anaphylactized animals would be lacking.

A very noticeable feature of the effect of the chloroform on the sera was the voluminous precipitate of the serum proteins produced. In this connection, the quantity of chloroform used seemed to exert no influence, those sera treated with but one-tenth their volume of chloroform yielding just as heavy precipitates as when treated with the larger quantities. Also, it made no difference whether the serum was on the machine being shaken constantly, or standing with only an occasional shaking. Time was of but slight consequence, the volume of the precipitate seeming not to increase after the first few minutes of its contact with the chloroform. The precipitates were white or nearly so, sometimes slightly tinged by hemolysis, and were granular to creamy in consistency. These precipitates consisted of the serum proteins. A filtered salt solution responded to the protein color tests, excepting the glyoxylic. A partial

precipitation could be induced by saturation with magnesium sulphate or half saturation with ammonium sulphate. When suspended in water and dialyzed for some time, the dialysate gave the xanthoproteic reaction, showing a water soluble portion. Dilute acids gave only a cloudiness, but strong acids produced marked coagulation, with solution upon excess and heating. The alkaloidal precipitants gave the usual precipitates from the dilute salt solution. It was concluded that the precipitates were the serum albumins and globulins.

It will be observed that in some cases the rabbit and rat sera when shaken without the addition of chloroform became appreciably toxic. In the case of the rat serum a visible agglutination of the serum had taken place, the rabbit serum sometimes showing a slight lack of clarity, but no definite coagulum. Undoubtedly the shaking produced some change in the dispersion of the serum contents that resulted in toxicity.

Keeping in mind the possibilities of error, the rabbit and the dog serum were tested, both before and after treatment with chloroform, for their iodine absorption. These two sera were considered in a measure as being representative of the two groupings mentioned earlier. A measured quantity of the serum was placed in a tube together with some chloroform to act as a solvent, and then a known iodine solution added. Controls were made of tubes without serum. The tubes would then be shaken for 15 minutes and the excess iodine titrated back with sodium thiosulphate. The iodine absorbed was calculated to grams per cubic centimeter of serum. Table XIX gives the results.

TABLE XIX.

Iodine absorption by treated and untreated serum.

SOURCE.	WHOLE SERUM.	TREATED SERUM.
Dog	.0056 gm.	.0048 gm.
Rabbit	.0068 "	.0061 "

It will be recalled that this work was based upon the assumption of anaphylatoxin production by the removal of lipoidal antitryptic substances by chloroform. The iodine absorption would indicate a decrease in the unsaturated bodies of the serum, but not their complete removal. In view of the vigorous treatment employed for their removal, assuming them to be soluble in chloroform, this conclusion is untenable. As an explanation for the disappearance of the iodine, it seems it could be accounted for on the grounds of adsorption by the serum colloids. The lack of distinctness in the end-point, so characteristic of iodine titrations, in this work, would indicate iodine in some rather loose combination with the serum bodies.

In conclusion I wish to express my thanks to Dr. V. C. Vaughan and to Prof. P. H. DeKruif for kindly criticisms and suggestions and to Mr. William German for material assistance in this work.

CONCLUSIONS.

I. The intravenous injection of high dilutions of chloroform may cause sudden death in guinea-pigs.

II. When blood serum has been shaken with chloroform the complete removal of the chloroform is difficult.

III. The toxicity of the serum falls with the completeness of the removal of the chloroform.

IV. Death in guinea-pigs caused by the intravenous injection of serum which has been shaken with chloroform, is often at least, not typical of anaphylactic shock.

V. Serum when shaken without chloroform may cause typical anaphylactic shock with death and typical autopsy findings.

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MICROSCOPIC STUDY OF THE FRESH BILE IN DISEASE OF THE LIVER*

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WITH the exception of Bondi,[1] who reports the finding of "Gallenzylinder" in the fresh bile removed by means of the duodenal tube (after a test meal) from a case of cirrhosis of the liver, we are not aware of any successful observations as to microscopical abnormalities in the bile of such cases. In one case of cholecystitis, also, the same observer noted numerous pus cells in the fresh bile. Petry[2] and others[3] have attempted, unsuccessfully, to obtain information from such studies. All have, however, so far as we are aware, attempted such studies after a test meal of some kind instead of obtaining the pure and fresh secretion undiluted by test meals of water, tea, beef juice, etc.

The series which we have studied consists of thirty cases of disease of the liver. In addition to these, however, about 120 other cases have been studied in a similar manner, these latter cases being either normal or suffering from some disease other than that of the liver. As a result of this work we believe that certain definite and abnormal microscopical changes can be detected in the bile in certain diseases of the liver and its appendages.

As to the technic which we employ, suffice it to say that the duodenal tube is passed upon a perfectly empty stomach, no fluids whatever being allowed for several hours preceding the test (12 hours). Another most important point, which must be observed, is the necessity for an immediate examination of the duodenal contents as soon as removed, since only a few minutes are required for either the complete destruction of the important cellular elements, or for such serious mutilation by the pancreatic ferments as to make them unrecognizable. It is necessary, moreover, to wait until the duodenal contents have become alkaline or neutral in reaction before making the microscopic examination, since the presence of an excess of acid will, by precipitating the bile salts, completely obscure the microscopic picture obtained in an alkaline medium.

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The microscopic examination is made in the same manner that fresh urine is examined for casts, etc. The fresh contents are placed upon a slide and studied first with a low power lens, then, as soon as a suspicious group of cells is noted, the details are studied with the high power. The oil immersion is not employed. The duodenal contents may be centrifuged. As a rule, however, we omit the centrifuging, since it takes time, contenting ourselves with several examinations of the uncentrifuged specimen.

The appearance of the normal duodenal contents, prepared in this way, is easily described, since practically no cellular elements are noted, except an occasional epithelial cell or a few leucocytes, from the stomach, or a squamous epithelial cell from the mouth or esophagus. Such cells are quite easily recognized by anyone with experience in the microscopic appearance of the gastric contents.

Abnormal microscopic findings in the duodenal contents may be divided, in our experience, into six classes: *First,* bile-stained, granular casts, often suggesting casts of the finer bile passages, occasionally containing a bile-stained cell.* *Second,* bile-stained leucocytes, usually polymorphonuclear, often occurring in groups, and often associated with the third abnormality. *Third,* a round, oval, deeply bile-stained, refractile cell about twice the size of a polymorphonuclear leucocyte, and containing a round, small and often eccentric nucleus. This cell is extremely fragile, disappearing from the field in a very few minutes, being replaced by deeply bile-stained, amorphous and highly refractile masses. Occasionally such cells are found in the form of small casts. *Fourth,* columnar, bile-stained epithelial cells, apparently from the linings of the gall bladder or gall ducts, often occurring in small masses. *Fifth,* the occurrence of large numbers of bacteria, yeasts or protozoa. Normally bacteria do not form a prominent picture in the fresh duodenal contents, although an occasional bacillus or a few cocci may be seen. Yeasts and protozoa are never found under normal conditions. In certain conditions, however, described later, the field may be teeming with animal life. *Sixth,* normally the pure duodenal contents are fluid, containing very little mucus, except that which comes from the stomach. Any marked increase of mucus, especially if associated with the cellular elements already noted, is of significance.

Of eight cases of advanced atrophic cirrhosis of the liver studied in the manner described, we found the first abnormality, amorphous bile-stained casts, in three. The second abnormality, bile-stained pus cells were found in three cases, and the round, refractile cells, deeply bile-stained were found in five cases. Three of these cases subsequently came to autopsy and one to operation, when the diagnosis was confirmed. Two cases of atrophic cirrhosis gave quite negative results on microscopic examination of the bile.

The next most fruitful source of study for us by this method has been four cases of cholecystitis, three of which were acute. In two of the acute cases bile-stained pus cells were found, in one case numerous, in the other case being very few. The round, refractile cell, described as the third abnormality, was found in all three of these cases. In one of these cases innumerable staphylococci were

*"Gallenzylinder" of Bondi (loc. cit.).

also noted; in another large numbers of motile bacilli formed a prominent part of the picture. In one of these cases, moreover, large numbers of non-bile-stained polymorphonuclear cells were noted.* The one case of chronic cholecystitis, with occlusion of the cystic duct, was quite negative.

The bile from five cases of syphilitic cirrhosis of the liver has not shown as many cellular changes as we might expect. In one case the large, refractile cells, above described, were fairly numerous, associated also with a few pus cells, which were deeply bile-stained. The four other cases were, however, quite negative, except that one case, after repeated examinations, finally showed a very few bile-stained polymorpho-leucocytes. None could be found by similar methods in the other three, however. The diagnosis in all of these cases was made from the clinical signs, the positive Wassermann reaction and the therapeutic test.

Four cases giving alcoholic histories, showing large and tender livers, and having negative Wassermann reactions, were studied. Only one of these cases showed any important cellular changes. In this case abnormalities one, two, three and four were noted.

Three cases of tropical liver abscess, studied by this method were quite negative.

One case of carcinoma of the liver was negative.

Four cases of chronic passive congestion of the liver were negative, except for the presence of a few columnar epithelial cells in one case.

One case of acute yellow atrophy of the liver, which ended fatally, was interesting from the fact that bile-stained pus cells were numerous, and were associated with innumerable bacteria (chiefly the colon bacillus, but containing a few cocci).

Two cases, finally, which we were unable to classify, were of interest. The first was a man of fifty, complaining of pain in the right upper abdomen. Physical examination negative except a liver generally enlarged and slightly tender. Wassermann negative, previous history negative. We found large numbers of trichomonas intestinalis in this case, without any cellular changes, however. The gastric contents were also negative microscopically, and contained no protozoa.

The other case, a man of fifty-five, giving a negative alcoholic history and having a negative Wassermann, was admitted to the wards complaining of abdominal pain and tenderness. The liver was large, hard and tender. Physical examination otherwise negative. Microscopic examination of the duodenal contents showed innumerable lamblia intestinalis. These protozoa were so numerous, in fact, that everything else in the field was obscured by them. A large number of pus cells were found in this case.

In our series of cases not suggesting disease of the liver, being either normal or complaining of some form of gastric disturbance, or suffering from other diseases, such as typhoid fever, arthritis, malaria, uncinariasis, cardiac disease, nephritis, toxic vomiting of pregnancy, etc., consisting of some 120 cases, we have noted an occasional abnormality, as follows: In three cases of chronic gastritis, all three of whom gave alcoholic histories, the large, yellow, refractile

*Duodenitis.

cells, above described, were found in greater or less number. In one of these cases, moreover, a case coming to autopsy, fortunately, a few bile-stained pus cells were noted. At autopsy no serious lesion of the liver was found, a rather extensive cloudy swelling being practically the only lesion. One case of hypersecretion and hyperacidity, complaining of the usual pyrosis and gastric distress showed a few of the same round cells in the duodenal contents. In two cases of subacute gastritis, both non-alcoholic, a few of the round, refractile cells were found. With these few exceptions, out of some 120 selected cases, complaining of all forms of gastric disturbances, no abnormalities, such as we have described, have been found.

Finally, we believe that the following conclusions as to the value of the microscopic examination of the fresh duodenal contents may be drawn:

First: In certain diseases of the liver, particularly in atrophic cirrhosis, definite abnormal cellular elements are found in the fresh bile in a certain percentage of cases. It is probable that these cells may be of diagnostic importance in this disease.

Second: Such cellular changes may be of diagnostic importance in certain cases of acute cholecystitis.

Third: Abnormalities in the fresh bile are rarely found in other forms of hepatic disease, such as syphilis of the liver, chronic passive congestion of the liver, abscess and carcinoma of the liver.

Fourth: We believe that such examinations as we have described offer the only satisfactory means of studying inflammations of the duodenum (duodenitis) satisfactorily.

Fifth: In order to obtain satisfactory microscopic pictures, it is absolutely necessary that the bile be examined *immediately* after removal, that it be alkaline or neutral in reaction, and unmixed with food or liquids.

	DIAGNOSIS.	GROSS APPEARANCE.	MICROSCOPIC APPEARANCE.
1.	Atrophic Cirrhosis	Turbid Bile +++	Numerous bile-stained pus cells. Hepatic ? cells
2.	" "	Turbid Bile ++	"Gallenzylinder" bile-stained pus cells ++
3.	" "	Clear. Bile +	Few Hepatic ? cells
4.	" "	Turbid Bile ++	Hepatic ? cells. Bile-stained pus cells (few). "Gallenzylinder."
5.	" "	Clear Bile ++	Negative
6.	" "	Clear Bile +	"
7.	" "	Clear Bile +++	"Gallenzylinder." Hepatic ? cells
8.	" "	Turbid Bile +++	Hepatic ? cells
9.	Cholecystitis (acute)	Turbid. Bile ++ Mucus ++	Few bile-stained pus cells. Bile-stained cylindrical epithelial cells.

	DIAGNOSIS.	GROSS APPEARANCE.	MICROSCOPIC APPEARANCE.
10.	"	Clear. Bile + Mucus +	Very few bile-stained pus cells
11.	" (typhoid)	Clear. Bile Scanty Mucus +	Bile-stained pus cells fairly numerous. Hepatic ? cells B. Typhosus.
12.	Cholecystitis (chronic) Cystocele	Bile + Clear	Negative
13.	Syphilitic Cirrhosis	Bile + Clear	Hepatic ? cells ++ Few bile-stained pus cells
14.	"	Bile scanty Clear	Few bile-stained pus cells after three trials
15.	"	Bile + Clear	Negative
16.	"	Bile + Clear	"
17.	Syphilitic Cirrhosis	Bile + Clear	Negative
18.	Large tender liver Alcoholic history	Bile ++ Turbid	"Gallenzylinder." Bile-stained pus cells. Hepatic ? cells.
19.	"	Bile +++ Turbid	Negative
20.	"	Bile +	"
21.	"	Bile +	"
22.	Hepatic Abscess	Clear Bile scanty	"
23.	" "	" "	"
24.	" "	" "	"
25.	Carcinoma Liver	" "	"
26.	Chronic Passive Congestion of Liver	" "	Cylindrical epithelial cells
27.	"	" "	Negative
28.	"	" "	"
29.	"	" "	"
30.	Acute Yellow Atrophy of Liver	Bile ++ Clear	Numerous bacteria (B. Coli and Gram-positive cocci) Pus cells ++
31.	Large tender Liver	Bile ++ Clear	Numerous trichomonds intestinalis
32.	" " "	Bile++ Clear	Pus cells ++ Innumerable Lamblia intestinalis

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LABORATORY METHODS

Newer Laboratory Methods for the Early Diagnosis of Pulmonary Tuberculosis

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IN the past decade quite a number of laboratory methods have been suggested for the early diagnosis of pulmonary tuberculosis. The various tests concern themselves with the sputum, the urine, the feces and the blood. Whereas bacteriological examination of the sputum is of absolute diagnostic importance, the other laboratory procedures have given rise to much commentation and dispute and are as yet only of relative value.

ALBUMIN IN THE SPUTUM.

Stimulus was given by Roger,[1] in 1909, to the question of albumin in the sputum of tuberculous patients, and it became the subject of considerable study. The occurrence of albumin in the sputum was mentioned by a few authors since 1855 as present in pneumonia and pulmonary edema,[2] but it has been shown that in these conditions extravasations of blood constitute the chief source of the albumin.[3]

The test is made as follows: Ten cubic centimeters of sputum are diluted with an equal amount of water. To this is added one cubic centimeter of glacial acetic acid to precipitate the mucin, and the mixture is filtered through fine filter paper. The filtrate shows albumin by boiling or by the nitric acid test.

The albumin has been supposed to indicate that the sputum is not a mere superficial secretion, but comes from some deep-seated inflammatory process.[4] Most of the recent investigators have asserted the belief that a negative test for albumin in the sputum contraindicated the existence of tuberculosis,[5] while a positive test rendered the case only suspicious, since a positive test may also be obtained in pneumonia, pulmonary edema, and heart disease with pulmonary congestion. It was later found that albumin was absent in fibroid tuberculosis.[6]

Some observers felt that an increase or decrease of albumin content of a tuberculous sputum points to aggravation or improvement of the pulmonary lesion, thus placing a prognostic significance to the findings. But Lockwood, in a very recent work,[8] examined the sputum of thirty cases of asthma, emphysema and bronchitis which had no evidence of tuberculosis. He used a one per cent solution of phosphotungstic acid, in alcohol for precipitation of the albumin. He found albumin in the sputum of all cases examined, tuberculous and nontuberculous. While the average quantity of albumin was found to be higher in the tuberculous cases, there was such a wide variation in the quantity in both the tuberculous and nontuberculous that this could not be used as a diagnostic aid. In the tuberculous patients, the amount seemed to bear no

relation to the stage, duration or activity of the disease process. Yet one deduction seems feasible from the results; i.e., the absence of albumin in the sputum may be deemed as confirmatory proof of the absence of tuberculosis.

FERMENTS IN THE SPUTUM.

The occurrence of ferments in the sputum for diagnosis attracted little study, though observations were made on the subject by Stolnikoff[9] and Escherich[10] about forty years ago. In the examination of tuberculous sputa by Eiselt[11] a proteolytic ferment, chiefly trypsin was found; during febrile periods the trypsin disappeared and was replaced by antitrypsin. No lipolytic ferments were found, but albumoses and peptones were present, probably from the occult bleeding which may have occurred. The ferment action, according to the author, was in inverse proportion to the content of coagulable albumin, and in direct proportion to the amount of albumoses and amino-acids in the sputum.

SALICYLIC ACID TEST.

Falk and Tedesco reported a test in 1909[12] which promised to differentiate disease processes limited to the bronchial mucous membrane from those which had extended to the lungs. The principle of the test was based on the assertion that salicylic acid when present in the blood, appears in inflammatory exudates from the lungs, but is not excreted from the bronchial mucosa. The patient is given thirty grains of salicylic acid and the sputum is collected for twelve hours. The sputum is then tested for salicylic acid by a solution of ferric chloride. A violet color indicates a positive reaction.

GLYCOGEN TEST.

Another observation that has been made on the sputum in pulmonary tuberculosis is the increased proportion of glycogen, reported by Moscati,[13] and confirmed by Pozzilli[14] and others. Moscati concluded from his studies of many cases, that glycogen always exists in increased quantities in tuberculous sputum, and that it is always increased with destructive lung processes. It forms 2 or 3 per cent in the advanced stages of the disease. Pozzilli tabulated the findings in twenty-two cases of various affections of the air passages. In one case of advanced pulmonary tuberculosis, glycogen formed 3.75 per cent of the sputum, and was never below 2.5 per cent in other advanced cases. In earlier stages of the disease, it ranged from 0.15 to 0.6 per cent. It was not demonstrable in simple bronchial catarrh or in acute bronchitis. In pneumonia, it ranged from 0.05 to 0.06 per cent. The highest proportion detected in any nontuberculous affection, 0.25 per cent, was in a case of putrid bronchitis. The proportion of glycogen may thus prove a valuable aid for differentiation of tuberculosis and for prognosis. The loss of glycogen must be one factor in the debility of consumptives and may also throw light on the peculiar susceptibility of diabetics to tuberculosis.

The glycogen can be demonstrated by the iodine test or by Pflüger's technic. For the former the sputum is rendered slightly alkaline with sodium carbonate, and then heated for an hour to allow the glycogen to dissolve out into the water. The fluid is then filtered and boiled down in the water bath. An

iodo-iodide solution (0.25 parts of iodine; 0.5 part of potassium iodide and 100 parts of water) is poured on the filtrate in a test tube held very slanting. A mahogany ring forms at the junction of the two fluids in the presence of even very small proportions of glycogen.

CYTOLOGICAL STUDIES.

Cytological studies of the sputum have been found to be quite typical in pulmonary tuberculosis. Attention was first called to an increase of a cellular element in the sputum as an early sign of tuberculosis by Wolff-Eisner in 1907.[15] He found an increase in small lymphocytes in early tuberculosis, anywhere from 33 to 90 per cent. The pathologic significance of lymphocytes in the sputum is difficult to explain, but it has probably to do with the relationship of the toxins of the tubercle bacillus to the emigration of leucocytes. A high lymphocytic content of the sputum is in itself strong presumptive evidence of tuberculosis. While conversely, a high polynuclear content speaks against it, except in cases of mixed infection, where, of course, tubercle bacilli are as a rule present in large numbers.[16]

Recent studies on the value of the test have confirmed the belief that a sputum lymphocytosis showing under the microscope 50 per cent or more of lymphocytes speaks strongly for the presence of pulmonary tuberculosis. It would seem desirable that a careful cytological examination should be made of every sputum that is negative for tubercle bacilli. If a positive albumin finding is present and a preponderance of lymphocytes, we may be justified in assuming that the source of this expectoration is a tuberculous focus in the lungs.[17]

UROCHROMOGEN TEST.

The chemical changes occurring in the urine during tuberculosis have been given considerable study, frequently with contradictory findings. The disparities among the results of different authors are probably chiefly due to the fact that it is difficult to select for such studies a group of tuberculous individuals wholly free from various conditions that influence the urinary output and thus complicate the problem.

A number of investigators, following Weiss, who published his findings in 1910,[18] have reported marked increase in urochromogen in the urines of tuberculous individuals. They have claimed that the amount of urochromogen output increased with the progress of the disease and that in general the amount of urochromogen suggests the degree of tissue destruction in the body.[19] In a number of cases reported, the quantity of urochromogen ran parallel with the other clinical symptoms of the disease, and always became negative in cases which could be considered clinically cured.[20] The occurrence of excess of urochromogen has therefore been advocated as of diagnostic as well as prognostic value in tuberculosis.[21]

The test is simple. Five cubic centimeters of urine are diluted with ten cubic centimeters of water. A few drops of a dilute (1-1000) permanganate solution are added. In the presence of excess of urochromogen, a deep yellow color appears. The test seems from its very nature, not specific for tubercu-

losis. The reading is sometimes made difficult by the deep yellow color of certain specimens of urine, and also because a number of normal urines become more yellowish after addition of permanganate. It sometimes occurs in normal urine and is quite constant in typhoid.

The original Ehrlich's diazo reaction has also been used in the diagnosis of chronic tuberculosis. It depends upon the formation of some unidentified coloring matter produced by the combination of some undiscovered substance in the urine with a diazotized aniline.[22] Usually a diazotized sulfanilic acid solution with a solution of sodium nitrate is added to an equal volume of urine. A red color forms on the addition of ammonia.

According to Weiss, the normal coloring matter of the urine is derived from a chromogen, which in its properties is similar to the proteic acids which are present in the urine. This is the substance he holds responsible for the Ehrlich's diazo reaction. When this substance is oxidized, either by dilute solution of potassium permanganate or by iodine and potassium thiosulphate it is changed to the true urochrome of the urine.

In 1914 comparative studies were made of urinary findings in two hundred cases.[23] Of these, one hundred were clinically tuberculous and one hundred clinically nontuberculous. Of the former, only forty-six or less than 50 per cent gave a positive Weiss and a positive diazo reaction, and forty-two were negative for both tests. Of the one hundred nontuberculous cases (excluding typhoid) thirteen urines were positive to both tests and seventy-eight urines negative to both. The authors reached the following conclusions:

The Weiss and diazo tests in tuberculosis are not constant, as there are various other pathological conditions in which these tests are positive. The urinary findings are not sufficiently frequent in tuberculosis to be of special diagnostic value, even when other possible complications giving rise to positive tests can be excluded.

These conclusions were corroborated in a more recent study by Bronfenbrenner on tuberculous cases which the author had under observation in his clinic.

TUBERCLE BACILLI IN STOOL.

A few years ago Meyer Solis-Cohen reported six cases in which tubercle bacilli were found in the feces before they were discovered in the sputum.[24] In several of the cases at the time, there were no symptoms or signs of intestinal tuberculosis. He therefore suggested routine examination of the feces for tubercle bacilli in cases of pulmonary or general tuberculosis, believing that frequently tubercle bacilli will be found in cases in which the sputum is negative or not obtainable. The importance of this test is obviously doubtful.

ARNETH CLASSIFICATION OF LEUCOCYTES.

Studies of the blood have evolved certain refinements in the differential counting of the leucocytes which may serve to assist in the diagnosis of early pulmonary tuberculosis.

Arneth classified the neutrophile polymorphonuclear leucocytes according to their nuclear division. He considered that certain polymorphonuclear cells, those with one or two nuclei are less resistant and less able to combat infec-

tion than those with more nuclear lobes.[25] He found from a study of the blood of fifteen healthy men that the normal picture was as follows,

I	II	III	IV	V
5%	35%	41%	17%	2%

giving an index of 40, counting the sum of classes I and II as the index. He also found that the number of lobes was independent of the total number of leucocytes. Minor and Ringer found a slightly different normal from a study of the blood of ten healthy men,[26]

I	II	III	IV	V
2.5	18.2	55.6	18.6	5.1

giving an index of 20.7 as the normal. Bushnell and Trueholtz take as their normal index the sum of I plus II plus half of III which would give according to Arneth 60 as the normal and according to Minor 48.5.

In tuberculosis Arneth found that there was an increase of those with single or two lobes and a decrease of III, IV and V. Thus in severe cases, counts were obtained in which there were 36 per cent of I, 56 per cent of II, 8 per cent of III and zero of IV and V. The occurrence of such a deviation to the left, as it is called, was confirmed by Klebs,[27] Bushnell and Trueholtz[28] and by Minor and Ringer.

The cause of this phenomena is unexplained, but the degree of this deviation from the normal appears to be roughly proportionate to the severity of the infection and may be taken as an estimate of the amount of toxemia. According to some authors such changes in the neutrophiles in tuberculosis offer a distinct aid in prognosis, and serve as a guide to treatment.[29]

We have made a study of the "Arneth" blood-picture in twenty-seven cases attending the tuberculosis clinics of the New York Health Department. The patients were in various stages of the disease. All showed tubercle bacilli in the sputum at the time of the blood examination.

Arneth used Ehrlich triacid stain. From his work, one gains the impression that he preferred a stain which would not stain the nucleus sharply in order that the threads connecting its subdivisions might not become visible. Bushnell used Delafield's hematoxylin and 0.5 per cent alcoholic solution of eosin, staining first with eosin, washing and then staining with hematoxylin. We used Jenner stain, which we found to give good nuclear differentiation.

To obtain percentages of the different types of cell, we first counted 100 white blood cells, giving directly the percentage of the various leucocytes. Then we counted the neutrophiles alone until we had classified 100 into their Arneth divisions, thus obtaining the percentages of the different classes below. In classifying the neutrophiles, it is at times difficult to say whether the nucleus has two or more lobes. We therefore adhered to the rule that if there is any band of nuclear tissue, except a thin chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purposes of the count, be said to be divided. (See Table I.)

The results in our cases agree closely with all other workers.[30] In the

more favorable cases, the number of neutrophilic cells of class I and II diminish, while in the severer cases, these cells increase in number. It is difficult to explain why Solis-Cohen and Strickler[31] obtained results directly opposed to the findings of all other workers, but there is one error obvious in their calculations. While considering their normal index as the combined percentage of neutrophilic cells with one and two nuclei, they make comparison of their index with that of Minor and Ringer and that of Webb and Williams who include half of III in their index count. In our tables, the latter index was calculated.

TABLE I.

STAGE.	I.	II.	III.	IV.	V.	POLYS.	LYMPH.	L. M.	EOS.	BAS.	INDEX.
	6	37	44	11	2	65	26	8			65
	15	40	35	9	1	50	37	11	1	1	72
I	25	49	19	6	1	45	46	2	5	2	82
including non-	6	27	47	16	4	56	36	4	4		56
toxic cases.	13	46	35	6		67	27	4	1	1	76
	2	52	36	12		73	25	2			72
	11	49	32	7	1	79	19	1	1		76
	19	57	24			54	37	6	3		88
	34	36	21	9		64	35		1		80
	29	33	30	7	1	56	32	6	6		77
II	22	40	33	4	1	71	27		2		78
including cases	14	48	31	7		66	24	9	1		77
with slight	45	30	24		1	57	34	7	2		87
fever.	12	46	38	3	1	66	27	4	3		77
	12	49	34	5		59	37		3	1	78
	9	55	31	5		85	15				79
	15	40	32	7	6	56	27	16	1		81
	24	48	21	7		78	18	3	1		82
	30	40	27	3		58	34	4	3	1	83
	58	29	12	1		78	20		2		93
III	60	32	7	1		56	39	2	2	1	95
including cases	49	39	10	2		61	23	14	2		93
with high fever,	36	51	12	1		78	17	2	1	2	93
night sweats	50	40	8	2		54	33	11	1	1	94
and marked	17	47	26	9	1	72	22	3	3		90
toxemia.	41	42	12	5		74	15	11			89
	28	54	16	2		74	17	7	1	1	90

BACTERIOLOGIC STUDIES OF THE BLOOD.

Some authors claim to have demonstrated tubercle bacilli regularly by a direct microscopic examination of the blood of tuberculous patients,[32] and frequently also by the inoculation of such blood into guinea-pigs and rabbits.[33] Other laboratory workers have failed absolutely to obtain such results.

Now, with regard to the microscopic examinations, there are so many sources of error, such as the presence of acid-fast organisms resembling tubercle bacilli in distilled and tap water and certain saprophytic bacteria as well, the close resemblance of the stroma of the erythrocytes, crystals of lecithin and cholesterin and of flakes of fibrin, that positive reports may be attributed to these factors. The method has proven of no value.

With regard to the inoculation of guinea-pigs with the blood from tuberculous patients, it has been shown that ten tubercle bacilli suffice to inoculate a

guinea-pig successfully[34] and some think that this can be accomplished by a single tubercle bacillus introduced intraperitoneally.[35] The most recent experiments have shown the test on tuberculous patients to be negative. Blood from forty-seven advanced cases of tuberculosis was injected intraperitoneally into guinea-pigs. Only one of the guinea-pigs who received blood from a patient who had previously had a tuberculin injection, developed tuberculosis. (Kessel, 1915.[36]) All of the other injections were negative. This method of diagnosis must therefore also be discarded.

Attempts have been made to apply the agglutinin reaction to the diagnosis of tuberculosis. Arloing and Courmont[37] used a homogeneous culture of tubercle bacilli transplanted from potato to broth. The authors claimed significant results for the reaction. Kinghorn and Twichell[38] repeated the studies by the same method. Clear fresh human serum was used, and was obtained either from the lobe of the ear or from a vein in the arm. With each serum, mixtures of different strengths were prepared, one part of serum to five, ten, fifteen, etc., parts of emulsion of broth culture of tubercle bacilli. When agglutination took place in a dilution of one to five within five hours the reaction was considered positive. The authors obtained 84 per cent positive reactions with the sera tested from healthy persons, and 87 per cent positive from tuberculous sera. There is therefore little, if any, difference between the agglutinating power of healthy and of tuberculous sera.

The precipitin and meiostagmin reactions, and lately the biochemical test of Abderhalden have also been studied for the diagnosis of tuberculosis, but the results were of scientific interest only.

COMPLEMENT FIXATION IN TUBERCULOSIS.

Following the demonstration of the value of the Wassermann reaction for the diagnosis of syphilis in 1906[39] many attempts have been made to adapt serologic methods to the diagnosis of tuberculosis.

The method of testing, is based on the phenomenon of Bordet and Gengou,[40] or fixation of complement, of which the Wassermann test is an atypical example. An antigen, such as the toxin of tubercle bacilli, when put in the presence of its specific antibody, which is supposed to circulate in the blood of tuberculous patients, will unite together, through the intervention of a third thermolabile substance which exists in all serums and is called complement. If one now puts into this triangular mixture of antigen-complement-antibody, a second antigen, say red blood cells and an antibody to these blood cells which will lake them, the red blood cells will not be laked if the complement which is also necessary here, has been used up in the first reaction.

The main reason why complement fixation in tuberculosis has failed seems to have been the lack of a suitable antigen. This gap seems at last to have been filled through the discovery of Besredka of a new method of cultivating tubercle bacilli.[41] With Besredka's method using a mixture of bouillon, egg-yolk, and egg-white, a luxuriant growth occurs within several days. The germs cultured in this way show biologic properties distinctly different from those observed heretofore, one of the most striking being the rapidity of their growth, which almost equals that of streptococcus.[42] To prepare the antigen, this culture is

heated and deprived of its cellular elements by filtration. The filtrate is titrated for anticomplementary or hemolytic properties, and then used as antigen. Craig[43] prepared his antigen similarly, using several strains of tubercle bacilli of the human variety grown in alkaline broth to which was added one dram each of egg-white and egg-yolk to 250 c.c.

The author has had the privilege of examining in collaboration with Bronfenbrenner, the comparative value of the Weiss test for neutral sulphur, the Ehrlich's aldehyde test and the Besredka serum test. Fifty-nine cases attending the tuberculosis clinics of this city were studied. The results, which will be published in detail elsewhere in a more comprehensive paper,[44] may be summarized as follows: The serum test was positive in about 92 per cent of the cases; while the Weiss test was positive in about 13 per cent of the cases, and the diazo test in about 8 per cent.

In first stage cases, the serum test was positive in 84 per cent, while the Weiss test was positive in 4 per cent; in second stage cases the serum test was positive in 94 per cent and the Weiss test was positive in 9 per cent, whereas in third stage cases, the serum test was positive in only 15 per cent whereas the positive urochromogen test rose to 31 per cent.

It is therefore to be concluded that the urinary findings do not go parallel with the serum findings. On the contrary, the latter were very high in the primary and secondary stages of pulmonary tuberculosis and fell very markedly in the tertiary stage of the disease, while the urinary findings seemed to show a very marked increase as the disease progressed.

Tuberculin of Besredka seemed to give the best results in diagnosis by complement deviation test. Even though the test was positive in a certain number of clinically nontuberculous cases, the reaction seemed to be specific. At least in 87 per cent of such cases, fixation was also obtained by one or more tuberculins other than that of Besredka, and such findings can well be attributed to the fact that in its earlier stages, the tuberculous process does not give symptoms sufficient for clinical diagnosis. The increase in the incidence of the Weiss reaction, together with the corresponding inverse frequency of occurrence of the serum test is quite constant in advanced stages of the disease, and may be of prognostic value, the former indicating tissue destruction, and the latter showing the lack of reacting power on the part of the body.

Lastly by a very simple method of preparing tuberculosis antigen by triturating tubercle bacilli in a mortar in salt solution, H. R. Miller[45] claims to have obtained 100 per cent positive results by complement fixation in positive cases of tuberculosis, and 100 per cent negative results in nontuberculous or clinically cured cases. The results are extremely interesting and require further confirmation.

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The Dissociation Curve as an Index to the Hydrogen-ion Concentration of Blood*

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In the study of clinical cases of dyspnea, often referred to as renal or cardiac asthma, more and more attention is being paid to the reaction of the blood. Now, the reaction, or better the hydrogen-ion concentration of the blood, may seem rather remote from the activity of the respiratory center, but consideration of the following physiological facts will show that indeed a very intimate relationship exists.

The cells of the organism have been so sensitized to their environment that, in order to carry on their normal metabolic activity, they require a circulating nutrient medium whose hydrogen-ion concentration remains practically constant. Hence the reaction of the blood and body fluids stands as one of the most constant facts in the whole organism. Since cellular activity demands this constancy of reaction, there must be some mechanism whose function it is to respond to slight alterations, and thereby rapidly restore the blood to its normal reaction. Such a mechanism is the respiratory center.

During metabolic activity there are added to the blood stream certain acid products—the so-called "fixed acids," which are eliminated slowly by the kidney, and a volatile acid CO_2, eliminated rapidly through the lungs. Obviously it is this latter mechanism that has to do with the rapid restoration of the blood to its normal reaction. When the blood becomes more acid, either from an increased production or a failure of elimination of these acid products, the respiratory center responds and attempts to reduce the acidity by increasing pulmonary ventilation. This increases the elimination of CO_2 and suffices to reduce the blood to its normal condition, e.g., in physiological exercise, where the acidity is largely due to an increased production of carbon dioxide. In certain pathological cases, however, where the acidity is due to the presence of nonvolatile acids, this mechanism does not suffice and the patient remains dyspneic.

It is seen therefore that in the behavior of the respiratory center there exists the most delicate indicator in the body for the detection of slight alterations in the reaction of the blood. In fact physiological experimentation shows that pulmonary ventilation is increased over 100 per cent by an increase in the hydrogen-ion concentration of the blood which is smaller than can be detected by any known laboratory method.

Next to the respiratory center in order of sensitivity to changes of hydrogen-ion concentration stands the chief respiratory agency of the blood, namely, hemoglobin. As pointed out by Barcroft and others, the affinity of hemoglobin for oxygen bears a constant and definite relation to the hydrogen-ion concentration of the serum. This affinity can be ascertained by exposing blood at body temperature to an atmosphere containing an accurately known pressure of oxygen. The blood is then examined in Barcroft's differential manometer, in order to find what percentage of the O_2 which it ought to absorb at a given O_2

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pressure has actually been absorbed (the so-called percentage oxygen saturation). The lower the percentage saturation, other conditions being unchanged, the higher the hydrogen-ion concentration of the blood.

To determine the oxygen saturation, the following method, devised by Barcroft[1] and slightly modified by Kato,[2] is the most useful for the study of clinical cases.

Description of Method.—One to two-tenths c.c. of defibrinated blood are drawn into the stem of a small serological pipette and allowed to flow into the bulbous portion. The neck is connected to the delivery tube of a Berzelius gas holder by means of a small piece of thick-walled rubber tubing. It has been found convenient to interpose a small wash bottle to gauge the rate of flow of the gas. The stem of the pipette is introduced into a three-inch piece of glass tubing, which is clamped to a wood block in a water bath—care being taken to have the bulb containing the blood well covered with water. The bath is kept at 38° C. by a thermo-regulator.

The pipette is kept in constant rotation either by hand or better by means of a small electric motor equipped with an eccentric. A cord is passed under the pipette just below the bulb. (A small bit of rubber tubing placed at this point will offer sufficient resistance to insure good rotation.) One end of the cord is attached to the eccentric, the other to an ordinary rubber band tied to an iron stand.

To prevent the entry of water into the tip when the pipette is immersed in the bath, we have used a suitable bit of rubber tubing over the end of the stem. The free end is plugged with a glass bead. Just before the introduction of the gas the tubing is elevated above the surface of the water and the plug removed. Again when the pipette is removed from the bath the bead is inserted to prevent the ingress of air due to contraction from cooling.

Rotation is continued for ten minutes, during which time a slow but continuous stream of gas is allowed to flow over the blood. The pipette is now removed from the bath. A little gas from the holder is now introduced in order to establish a slight positive pressure within the bulb, so that when the tubing over the end is removed there is no ingress of air. The pipette is held upright and the blood forced down into the stem by compressing the rubber tubing connected to the neck, this tube having previously been clamped and disconnected from the gas holder. The blood is delivered under dilute ammonia water contained in the gas bottle of the Barcroft differential manometer and its percentage saturation determined as described by Barcroft.

Normal blood gives a percentage saturation of 69 to 74 per cent at 17 mm. O_2 pressure in the absence of CO_2, that is to say, when human blood is exposed to an atmosphere containing 17 mm. O_2 at body temperature, 75 per cent of the hemoglobin is oxyhemoglobin and 25 per cent reduced hemoglobin. If this figure is lowered (e.g., to 50 per cent, so that 50 per cent of the sample is oxyhemoglobin and 50 per cent reduced hemoglobin), it indicates a certain increase in the hydrogen-ion concentration of the blood.

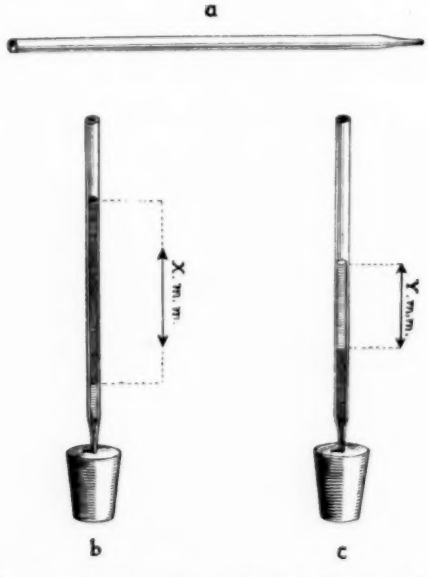
[1]Barcroft: Respiratory Function of the Blood.
[2]Kato: Jour. Physiol., Vol. 1, 1.

A Simplified Hematocrit and A Method For Determining Variations in Blood Volume*

By Albert A. Epstein, M.D., New York City, N. Y.

THE apparatus (see diagram, Fig. a) consists of small glass tubes made from selected glass tubing of uniform bore (0.5 to 1 mm. in diameter and 6 to 8 cm. long) drawn out at one end into a fine capillary. The blood is obtained from the finger tip (lobe of the ear or vein) in the usual manner. Clotting of the blood is prevented by means of finely powdered hirudin.

Just before drawing the blood, the wide end of the tube is dipped into the powdered hirudin and withdrawn. A few granules of the substance (usually sufficient to prevent clotting of the blood) adhere to it. After the finger is pricked and the blood oozes out easily and without pressure, the end of the tube containing the hirudin is brought in contact with the blood which flows readily



Formulæ for computing percentage volume of plasma and blood cells.

(1) Per cent of plasma $= \frac{y}{x} \times 100.$

(2) Per cent of blood cells $= \frac{x-y}{x} \times 100.$

into the tube. The tube is half filled with blood, and is then tilted two or three times to permit the hirudin to mix with the blood. Holding the tube slightly inclined with the capillary end downward, the blood is allowed to run down to about one centimeter from the capillary end, which is then sealed in a flame. A minute test tube is thus formed. The column of blood (which remains separated from the bottom of the tube by a cushion of air) is then measured by means of a millimeter scale and its length carefully determined. The sealed end of the tube is then inserted into a cork (see diagram, Fig. b) (which has a small hole

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in it) and centrifuged for ten minutes at the rate of about 2,500 to 2,600 revolutions per minute. The cells are completely sedimented.* The column of supernatant plasma (see diagram, Fig. c), which is clear and sharply separated from the cells, is measured by means of the millimeter scale and its length determined. The difference between the column of plasma and the original column of blood represents the volume occupied by the blood cells. The value of both plasma and cells may be computed in terms of per cent by dividing the figure obtained for each of them by that of the original column of blood, and the quotient multiplied by 100.

Example:

$$\frac{\text{Column of blood cells in millimeters} \times 100}{\text{Original column of blood in millimeters}} = \text{Volume of blood cells in the blood in per cent.}$$

$$\frac{\text{Column of plasma in millimeters} \times 100}{\text{Original column of blood in millimeters}} = \text{Volume of plasma in the blood in per cent.}$$

A different tube of the type described above is used for each determination of the relative content of cells and plasma in the blood. The accuracy of this method of measuring the blood cells and plasma is confirmed by the fact that in duplicate and triplicate determinations the results agree, although the amount of blood used in the several determinations need not be exactly the same.

Changes in the blood volume can be ascertained by means of repeated determinations of the relative amounts of plasma and cells present in the blood. It can be safely assumed, for reasons which need not be stated here, that the actual number of cells in the blood stream remains practically constant from hour to hour or even from day to day. A decrease in the proportion of blood cells in a unit of blood therefore signifies an addition to the fluid portion of the blood and hence an increase in the total volume. If the proportion of blood cells found at the beginning of any test or experiment be taken as the standard for comparison, the subsequent increase or decrease in the blood volume can be ascertained by dividing the percentage of blood cells in a given determination into the percentage found at the outset.

An increase in the proportion of plasma to cells signifies that the blood has become diluted and that the total volume of blood is therefore increased. Conversely, if the plasma is diminished and the cells per unit of blood increased, the blood is concentrated and its volume is diminished. The method of computation of the relative blood volume is illustrated in the following example:

The first examination shows:	Blood cell volume	= 50 per cent
	Plasma volume	= 50 per cent
The second examination shows:	Blood cell volume	= 45 per cent
	Plasma volume	= 55 per cent

Then the relative blood volume at the time of the second examination, is to the

*By using hirudin as an anticoagulant the blood cells settle to the bottom with extreme ease, and centrifugalization for a longer period than ten minutes at the above rate of speed, does not alter the final measurements and is therefore not necessary.

blood volume at the time of the first examination, as 45 : 50 = 100 : X, or $\frac{50 \times 100}{45} = X = 111.1$. In other words the relative blood volume at the time of the second examination is 111.1 per cent of that of the previous one. If, on the other hand, the first examination shows the cell and plasma relations as above, and in the second examination the cells amount to 55 per cent of the total blood, the relative blood volume at the time of the second examination, is to that found at the first examination, as 55 : 50 = 100 : X; or $\frac{50 \times 100}{55} = X$, i.e., X = 90.9 per cent.

In the first instance we find an increase, and in the second a diminution of the relative blood volume. Work is in progress which shows the necessity of controlling the quantitative findings of many ingredients of the blood by parallel estimations of variations in the relative blood volume.

The following protocols are supplied to illustrate the results obtained by this method:

TABLE 1.

Case L. K., I-4. Nov. 16, 1914. Coffee and glucose 50 g., 9:20 A. M.

Diagnosis: Hypopituitrism with diabetes.

Time	Blood cells Per cent	Plasma Per cent	Relative blood volume Per cent
9:15 A. M.	55.7	44.3	100.0
10:30	55.6	44.4	100.2
11:30	54.2	45.8	102.7
12:30 P. M.	55.5	44.5	100.4
1:30	55.5	44.5	100.4
2:30	55.7	44.3	100.0
3:30	55.7	44.3	100.0

TABLE 2.

Case L. F., K-16. Jan. 18, 1915. Coffee and glucose 50 g., 9:00 A. M.

Diagnosis: Diabetes mellitus.

9:00 A. M.	41.6	58.4	100.0
10:00	44.6	55.4	93.2
11:00	44.2	55.8	94.1
12:00	43.7	56.3	95.2
1:00 P. M.	44.3	55.7	94.0
2:00	43.5	56.5	95.6

TABLE 3.

Case A. M., K-1. Dec. 24, 1914. Coffee and glucose 50 g., 10:30 A. M.

Diagnosis: Diabetes mellitus.

9:30 A. M.	50.0	50.0	100.0
10:30	44.2	55.8	113.1
11:30	44.5	55.5	112.4
12:30 P. M.	44.0	55.0	113.6
1:30	42.6	57.4	117.4
2:30	42.9	57.1	116.5
3:30	48.0	52.0	104.2

TABLE 4.*

Cat 9.

Weight 3,750 gms. Complete pancreatectomy.

Time Hours	Blood**		Relative blood volume
	Plasma Per cent	Cells Per cent	Per cent
Before operation	64.3	35.7	100.0
After "	65.2	34.8	102.6
16	72.1	27.9	127.8
21	69.2	30.8	115.8
26	73.5	26.5	134.7
42	73.4	26.6	134.2
52	76.3	23.7	150.6
70	74.7	25.3	141.1
96	72.1	27.9	127.9
100	75.6	24.4	146.3
119	73.1	26.9	132.5
124	74.4	26.6	134.2
141	73.6	26.4	135.2

*Epstein, Albert A., and Baehr, George: Jour. Biol. Chem., 1916, xxiv, No. 1.
**Blood taken from ear vein.

TABLE 5.*

Cat 20.

Fasted 9 days before operation. Initial weight 3,300 gms. Final weight 2,880 gms. Pancreatectomy and double nephrectomy.

Before operation	62.3	37.7	100.0
After operation	58.0	42.0	90.0
½	56.7	43.3	87.8
7	65.2	34.8	108.3
18	70.0	30.0	126.0
23	74.2	25.8	146.1
27	74.5	25.5	146.0
30	76.0	24.0	157.0
41	76.0	24.0	157.0
46	77.2	22.8	165.1
48	77.1	22.9	165.0

*l. c.

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EDITORIALS

Recent Organic Arsenic Compounds

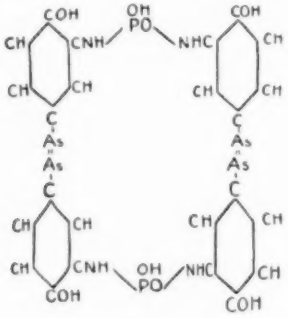
A STRIKING example of the great benefit which may result to practical therapeutics by the careful chemical, pharmacological and clinical investigation of new drugs is afforded by the recently introduced organic arsenic compounds. From the chemical standpoint certain peculiarly interesting and important features are manifested by these bodies. The first of these is the varying toxicity and often widely different symptoms which these bodies exhibit when compared with the results produced by corresponding quantities of arsenic combined in the inorganic compounds. There would seem to be involved here a peculiar combination of the action of the drug on the animal organism, and again of the reactions of the metabolic processes of the body on the drug. When parasitic organisms, such as spirochetes or trepanosomes, are present, as is usually the case in the conditions under which these drugs are administered, the conditions are still further complicated by the possible destruction of these organisms with the resulting liberation of toxic substances from this source. It is on this latter basis that a hypothetical explanation of the so-called Herxheimer (or Jarisch-Herxheimer) reaction which sometimes follows the administration of organic arsenic compounds in lues has been ex-

plained. In this condition there is swelling, edema, increased coloration or secretion, etc., about the local manifestations of the disease.

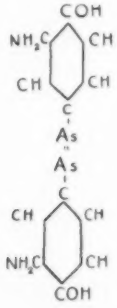
In most of its compounds the valence of arsenic is either three or five. And it seems probable that only the compounds of arsenic with oxygen[1] are capable of modifying vital functions. There are two oxides, the trioxide (As_2O_3), and the pentoxide (As_2O_5) of which the former possesses by far the greatest pharmacological action. This appears to depend on the trivalent arsenic atom. In the tissues of the body is it probable that each oxide is changed to some extent into the other, this being effected by reduction or oxidation of the pentoxide or the trioxide respectively. The ordinary salts of the acids of which these oxides are the anhydrides possess a corresponding action. In the tissues apparently the therapeutically effective arsenic exists in the form of arsenite ions, i.e., the trivalent form. This probably holds good as a general rule for the organic compounds also, for it was shown by Ehrlich that trypanosomes are not especially susceptible to the poisonous action of atoxyl in the test tube, but in the body atoxyl is very poisonous to these organisms. Ehrlich believed that in the tissues atoxyl was partially reduced and that these reduction products were the real trypanosome poisons, for he was able to show that such reduced bodies acted very powerfully on trypanosomes in the test tube. Others, however, are of the opinion that part of the atoxyl undergoes decomposition in the tissues and the ordinary inorganic arseniteion is liberated and thus exerts its usual action. A further peculiar and therapeutically unfortunate circumstance in the administraton of these arsenic compounds is that organisms such as spirochetes or trypanosomes tend to acquire a tolerance for the arsenic and, after a time, are no longer affected by therapeutic doses of the drugs. This appears as a rule to be more generally true in trypanosomiasis than in lues, for while the drug (atoxyl) may alleviate the symptoms of the disease, the cure of a patient with sleeping sickness is very rare.

The profound influence on humanity which a drug capable of curing such a disease as lues or trypanosomiasis may have has caused the greatest interest to be centered in the production and examination of organic arsenic compounds. And in addition, these drugs often appear capable of curing or alleviating diseased conditions other than those which were in the beginning the main objects of organic arsenic medication. Frambesia,[2] recurrent fever, Vincent's angina and spirillosis of the lower animals may be greatly benefited or cured by salvarsan, etc. And numerous favorable reports are in the literature regarding the use of these compounds in still other conditions.

A new impetus toward the chemical production of organic arsenic compounds has been given by the war, which has cut off from many countries the usual supply of salvarsan, neosalvarsan, etc. Accordingly a number of comp-onds, some new, others claiming to be the same as salvarsan, have appeared on the market. Among these are products from France, England, Canada, the United States, etc. Galyl[3] is claimed to be tetraoxy-diphospho-amino-diarsenobenzene and to possess the following formula:



The formula of salvarsan is as follows:



In galyl there are thus introduced two phosphoric groups. It contains 35.3 per cent of arsenic and 7.2 per cent of phosphorus, is a yellow powder of a tint different from those of salvarsan and neosalvarsan, and is insoluble in distilled water. It, however, dissolves rapidly in a weak solution of sodium carbonate, from which it can be precipitated readily by the addition of an acid. The tubes in which it is dispensed contain the small amount of sodium carbonate required for solution, and thus the handling and dissolving are no more complicated than in the case of neosalvarsan. Foerster believes this drug to be as good as salvarsan in the treatment of lues, but admits that his observations have not yet extended over a sufficient time to permit a final judgment on the substance. Some disagreeable after effects often followed the use of the drug, but these were not severe.

Kharsivan and neokharsivan[4] (English) are claimed to be identical with salvarsan and neosalvarsan as is also novarsenobenzol "Billion" (made by Pulenc Frères, Paris). There seems to be a general feeling among those who have used these drugs that they are somewhat more toxic than the original substances they were expected to replace. They may, however, with proper care be safely used.

Diarsenol as manufactured by the Synthetic Drug Company of Toronto, Canada, is said to be the dichlorhydrate of dioxydiamido-arsenobenzol. Apparently it differs only slightly from salvarsan. A number of reports[5] have been made concerning the action and use of this body.

"Arsenobenzol" (Salvarsan) has been manufactured by the Department of Dermatological Research of the Philadelphia Polyclinic.[6] This body (Arsen-

phenol-Amine Hydrochloride) is practically identical with salvarsan, but is slightly less soluble than the German product and requires to be filtered. It is prepared otherwise in the same manner as salvarsan and must be neutralized by the addition of sodium hydroxide solution. Ormsby[7] and Mitchell have recently contributed an interesting report of 184 injections of this drug in 75 patients. They conclude that arsenobenzol, together with mercury, offers as good a method of treatment of syphilis as any heretofore used.

A number of other organic arsenic compounds[8] have been introduced recently, but from published reports it is usually impossible to obtain accurate information regarding such therapeutic value as they may (or may not) possess.

It is easily seen that the ideal has not yet been reached in the production of these compounds. For aside from the difficulty of solution or of neutralization, etc., which several of them possess, there is the much more dangerous feature of their oxidation over into exceedingly poisonous bodies. In the case of the salvarsan and neosalvarsan there may be produced the dangerous paramidophenolarsenic oxide. This oxidation occurs rapidly in the air. The ideal drug should be stable, readily soluble, neutral in reaction, cheap and of low toxicity for the host while acting strongly on the parasites. It is to be hoped that the notable success of the past will continue to encourage greater efforts along these very promising lines in the future.

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—*D. E. J.*

Sensory Pathways in the Spinal Cord

INVESTIGATION of the exact function of the various tracts and centers that go to make up the complicated structure of the cerebrospinal axis is probably the most difficult problem in the whole domain of experimental physiology. The brilliant achievements of the morphological neurologists in tracing the origin and distribution of many of the tracts has not as yet been followed, to any material degree, by experimental evidence which would indicate the nature of the nerve impulses transmitted through them. Provided the function of the centers with which a certain tract connects is known, we can, it is true, draw inferences regarding the general nature of the impulses which must pass along it, but such inferences cannot be expected to carry us far when we consider, on the one hand, the paucity of our knowledge concerning the functions of the centers themselves, and, on the other, the diverse routes which may

be followed by the different groups of fibers that compose a given tract. Even if we could unravel to its last neurone the enormously complicated tangle of reflex arcs which compose the brain and spinal cord, we should know but little concerning the pathways along which different impulses travel. Thus, a pinprick on the sole of the foot will cause flexion of the homolateral hind limb (flexion reflex), whereas suddenly applied mechanical pressure at the same place will cause exactly the opposite effect, that is, an extension (extensor thrust). Both are reflex responses due to impulses traveling along practically the same reflex arcs, and yet the nature of the stimuli transmitted to the musculature of the leg is diametrically opposite in the two cases. Why the difference? It cannot be explained on any anatomical basis, and must therefore depend on conditions acting in the spinal cord in such a way as to direct and modify the entering sensory impulse so that it may either stimulate or inhibit the nerve cells of the motor neurones which, through the efferent roots, then transmit the impulses to the muscles concerned. In other words, to know the course of every nerve fiber and the position of every nerve cell in the cerebrospinal axis would help us no more in determining through which reflex arcs any given afferent impulse will travel than would an exact plan of a telephone exchange indicate the connections that are most frequently made between its incoming and its outgoing wires.

In order to ascertain the nature of the impulses which a given tract transmits, or a center originates, we must study either the motor and sensory paralysis which supervenes upon its destruction or the movements and sensory impression produced by its artificial stimulation. The stimulation method is practically limited in its applicability to the surface of the brain, where, however, it has been used with great success in the localization of the so-called motor and sensory centers. To determine the function of the more deeply situated centers and tracts, the "method of destruction" is alone available, but even this has only a limited applicability, for although it can be used quite successfully in dumb animals to determine the course of motor impulses, it cannot obviously tell us much regarding the sensory pathways.

Practically all the knowledge we possess regarding the course of sensory impressions has so far been gained by accurate clinical observation of patients in whom a subsequent pathologic study has been made of the exact position of the lesions. But this method, besides being time-consuming, cannot carry us far, and when we come to investigate the problem on laboratory animals, we are limited to sensory impulses which cause some visible or objective response; something we can see and measure. Pavlov during the last years of his life employed for such a purpose the secretory activity of the salivary glands, and recently Ranson and von Hess have published the results of some excellent experiments in which reflex changes in arterial blood pressure were observed before and after destroying certain of the afferent pathways in the spinal cord. In these experiments, also, evidence was collected regarding the influence of the lesions on the conduction of painful or noci-ceptive impressions, this being the only sensation which, by the behavior of the animal, we can know to be present or absent.

Various lesions were produced on the spinal cord of anesthetized cats at

the level of the first lumbar segment, and after recovery from the immediate effects of the operation "each cat was subjected to two kinds of tests to determine the effect of the lesion, first, on the conduction of pain and, second, on the character of the vasomotor reflexes." The lesions consisted of dorsal and lateral hemisections, section of the posterior funiculus and destruction of the apex of the posterior horn. After recovery from the immediate effects of the operation, there was no evidence of motor paralysis (except in one case). The sense of pain as indicated by a sharp cry and generalized struggling movements was tested, usually by means of faradic stimulation through needle electrodes, in the area of distribution of the sciatic nerve. After inserting the necessary cannulæ and while under light ether anesthesia, the vascular reflexes were elicited by faradic stimulation of the central end of the cut sciatic nerve, a pressor effect being obtained by strong and a depressor effect by weak stimulation. The influence of the spinal lesions on the reflex was gauged by comparing the magnitude of the change in blood pressure produced when the sciatic nerve was stimulated with that produced by stimulation of the central ends of the nerves of the anterior extremity. After these observations had been recorded, the animal was killed, and in order to determine the extent of the lesion a stretch of cord containing the lesion was removed, prepared by the pyridine-silver method of Ranson and cut into serial sections. By this method the presence of nonmedullated, as well as medullated nerve fibers, is revealed. In considering their results, the authors are careful to point out that all of the alterations in the vasomotor reflexes which were observed to supervene upon the spinal lesions which they produced cannot as yet be readily explained. The importance of their work lies, indeed, not so much in the actual conclusions which it is permissible to draw from the results, as in the great care and thoroughness with which the data have been collected.

Concerning the vascular reflexes, it was found that after lateral hemisection of the lumbar cord there occurred a great reduction of the depressor reaction elicitable by stimulation (weak faradic) of the opposite sciatic, whereas this reflex was practically normal when the sciatic on the same side as the cord lesion was stimulated. In the case of the pressor response, on the other hand, reduction was evident when either sciatic was stimulated, though more markedly so on the side of the lesion. These results suggest that depressor and pressor impulses travel in the spinal cord by different paths, of which the depressor are crossed and the pressor bilateral. Furthermore, the pressor paths are in the posterior portion and the depressor in the anterior portion of the cord, for in other animals in which posterior hemisections had been made it was found that the pressor reflex, but not the depressor, disappeared. The pathways available for the transmission of the pressor impulses in the posterior portion of the cord are the posterior columns (postero-median and postero-lateral) and the structures composing the tip of the posterior horn (Lissauer's tract, Substantia gelatinosa Rolandi, etc.). It was definitely established that it is by the apex of the posterior horn that the pressor impulses travel, for bilateral destruction of the posterior columns had no effect on the pressor reflex, which however could not be obtained after the apices of the posterior horns had been destroyed.

In connection with the last mentioned observations a most curious and in-

teresting result was obtained, namely, that the depressor reflex became very marked when the sciatic nerve was stimulated with such stimuli as would have caused a pressor response in a normal animal. This reversal of the normal reaction was also obtained, after destruction of the posterior horn, when the central ends of the brachial nerves were stimulated. To account for this result it is suggested by the authors that vasomotor centers, wherever these may be situated, are constantly being acted upon by afferent impulses which travel to them by different pathways, those coming up the lateral columns exercising an inhibitory or depressor influence, and those arriving by the posterior horns an augmentory or pressor influence. In normal animals, vasomotor reactions represent a balance between these two opposing influences. The facility of passage (threshold) of the depressor path is greater than that of the pressor, hence weak stimuli in normal animals cause depressor responses. Stronger stimuli, on the other hand, cause impulses to be set up in both pathways, but those with a pressor effect overcome the depressor impulses, so that the usual effect is a rise in blood pressure. The doorway guarding the depressor pathway is small and easily forced open, whereas the pressor doorway is large and unwieldy, but once forced open it offers less resistance to the crowd of impulses seeking passage along the pathway it guards.

After bilateral destruction of the posterior horns, "the pressor path is so impaired that with strong stimulation the inhibitory impulses along the depressor path predominate and produce marked depression." To explain why depressor responses should also be obtained in these animals on strong stimulation of the brachial nerves, it is suggested "that the normal high resistance of the pressor path has been increased above the lesion by cutting off the impulses normally ascending in this path from the lower half of the body."

The depressor pathway seems to be that along which painful impulses and those that cause reflex changes in respiration are conducted; at least, it could be definitely shown that the pressor pathway is not concerned in the transmission of such impulses, for none of the above mentioned lesions had any noticeable effect on the conduction of pain. This conclusion is in conformity with the usually accepted one that pain is transmissible up the cord in the anterior part of the lateral funiculus.

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—*J. J. R. M.*

Acidosis in Nephritis

HALDANE and Priestly showed that the regulation of respiration is effected by the carbon dioxide tension of the arterial blood which is the same as that of the alveolar air of the lungs. Later work showed that the hydrogen-ion concentration due to the dissolved carbon dioxide is the actual exciting agent. An increase of 0.22 per cent of the carbon dioxide content of the arterial blood results in doubling pulmonary ventilation. But carbon dioxide is

not the only substance which acts in this way, as has been proved by various investigators. Minute additions of hydrochloric acid will produce similar results. Lactic acid and undoubtedly other acids will act in the same way.[1]

While it is the lungs which are the chief organs for the regulation of the carbon dioxide content of the blood, it is the excretory organs, which govern the excretion of other acid products of metabolism. Bayliss remarks that the sensibility of the kidney to acid in the blood must be such as to keep the concentration of hydrogen-ion in the blood, other than that due to carbon dioxide, at a constant level.

Dyspnea may therefore be of one or another of two types,—that due primarily to increased carbon dioxide tension in the lungs and blood, and that due primarily to increased content of other acids in the blood. It is this latter type of dyspnea which led Peabody to study the acidosis of nephritis.[2]

Recent studies upon the blood in uremia have tended to show that this condition is frequently, especially in its advanced stages, associated with an acidosis which is sufficiently marked to alter the normal composition of the blood and to reduce the carbon dioxide tension. In chronic nephritis without uremia, investigations of the blood and alveolar air have shown little evidence of acidosis. That there is however a certain degree, often small, of acidosis in ordinary grades of nephritis, has been shown by Sellards,[3] who used what is known as the alkali tolerance test. (This test, credited to both Sellards and Palmer,[4] depends upon the fact that in a normal individual 5-10 grains of sodium bicarbonate taken by mouth causes a change in the reaction of the urine from acid to alkaline in about 2 hours. The test is applied by giving a patient 5 grains of sodium bicarbonate every 2 hours until the urine becomes alkaline to litmus. The amount of the bicarbonate necessary to produce the urinary change indicates the degree of the acidosis.) When the acidosis rises above a certain grade it is indicated by dyspnea, which is the physiologic expression of stimulation of the respiratory center.

Peabody notes that he and other workers have discussed the association of acidosis and dyspnea in cardiorenal disease, but that they have not been able to make clear the relation between the two symptoms. He remarks that the usual clinical laboratory examinations of the urine are not satisfactory because they give information only of the acid substances which are being excreted, and give no information as to how much is retained. This information may be had by making blood acidity studies supplemented by estimations of the carbon dioxide tension in the lungs. However that may be, all the *necessary* data may be had by the use of alkali tolerance test, which naturally will tend to show how much excess of acid there is within the body.

Peabody compares three groups of cases. The first consists of patients who gave evidence of a definite but not severe nephritis, as shown by urinary findings with, usually, hypertension and cardiac hypertrophy. In this group the alkali tolerance, phenolsulphonphthalein output, and alveolar carbon dioxide tension fell with normal limits. In other words, in this series of mild chronic nephritis there was no evidence of acidosis. The second group comprised cases in which the phenolsulphonphthalein output and nitrogen retention determinations were abnormal. In these the alveolar carbon dioxide determination were

normal but the alkali tolerance test required more than the usual alkali. In this series it is noted that the alkali tolerance and the output of phenolsulphonphthalein run parallel. These findings signify an early stage of acidosis without increase of alveolar carbon dioxide tension. The third group consisted of advanced cases of chronic nephritis. Six of the nine died within a few days or weeks of the observations. All the tests indicated low functional activity (phenolsulphonphthalein) of the kidneys. Alkali tolerance was increased and the alveolar carbon dioxide tension was decreased. In these cases, says Peabody, there is not only evidence of loss of base from the tissues, but also of increase of acids in the blood. These facts, he says, point to the acidosis of renal disease, being due to retention, or to inefficient secretion.

This work is exceedingly interesting and calls attention to important diagnostic and therapeutic procedures which have been dwelt upon very logically and impressively by Martin Fischer.[5] But Peabody's results do not go back far enough. That the acidosis is one due to retention is interesting, but not so interesting or important as the cause of the retention which Fischer has studied. In plain uncomplicated nephritis, there is edema of the kidney due to a local renal acidosis. Excretion may be cut down or not, and be a serious factor or not, according to the degree of renal involvement. When the acidosis of nephritis is more general then the edema is also more general and other tissues than the kidney become affected,—acids are held by all the organs according to the character of their arterial blood supply. When the brain becomes edematous during a nephritis, uremia results. In other words, isn't the cause in all cases much the same, and do not the symptoms vary with the organs involved?

That acidosis is but one factor in uremia is very probable, but also it is probably the most important single one, as alkali therapy indicates. It is noted that by alkali therapy Peabody means the administration of moderate intravenous doses of 5 per cent sodium bicarbonate solution. Such a solution does practically but one thing; i.e., it combines acid. When one considers what is happening in the acidosis of uremia, one must realize that the problem is not entirely one of acid content of the tissue though that is the primary thing, but it is water holding by the tissue. To reduce that needs more salt content, even in uncomplicated cases. In nephritis it is possible that the acids are not however the usual ones such as lactic acid and the rest, but they may be amino acids and to counteract the effects of these the alkaline salt solutions are not as efficacious as they are in the more usual types, in which they produce "great temporary improvement." In the group "in which acidosis only produces a slight fall in alveolar carbon dioxide tension, and in which there are no symptoms directly due to the acidosis, the results obtained by giving alkali are much more questionable," probably because of the type of acidosis. There is every probability that in the amino-acid acidosis alkali treatment is not as essential as sugar treatment. In other words, it is possible that by using a salt-alkali treatment on the one hand, or a dextrose or lactose containing salt-alkali treatment on the other, that the two types of acidosis might be separated.

It seems worth the saying that in very many chronic nephritides the vascular lesions are the important ones. In many cases of "uremia" the cerebral vessels are essentially diseased. When this is true, it becomes an important therapeutic

question to determine how much salt or alkali or other substance can be introduced to at once decrease the swelling associated with increased acid content, and to neutralize the acid. Neutralizing the acid in the blood alone will give only a transient effect. If the conditions are such that without a decreased carbon dioxide tension in the alveoli there is nevertheless a cerebral or a renal acidosis (swelling), it means that the available oxygen of the blood does not reach the organs, and, under such conditions, intravenous medication will always be only an experiment.

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—*P. G. W.*

The Bacillus of Plotz and Typhus Exanthematicus

THE repeated outbreaks of typhus fever which have marked the epidemic history of the present war promise to furnish the opportunity for final settlement of the problem of the etiology of this disease. Systematic scientific investigation of typhus dates from 1909 when Nicolle succeeded in inoculating anthropoid apes. Very soon after this, Anderson and Goldberger, Ricketts and Wilder, and Gavino and Girard successfully inoculated lower monkeys, and material for systematic experimentation was thereby made available. The definite temperature reactions in guinea-pigs inoculated intraperitoneally with typhus blood in the early stages of the disease were then reported by Nicolle Corneil and Conor, and this has been extensively confirmed by Anderson and many others, Anderson carrying a typhus virus in this way through many generations. The filtrability of the virus, though at first claimed by many observers and still held by some, was gradually shown to be unlikely by Anderson and Goldberger, Ricketts and Wilder, and, although occasional monkeys inoculated with filtered virus have proved refractory to subsequent inoculation, the general opinion today is that the disease is not caused by a virus too small to pass the filters. This of course has given new impetus to the search for bacterial causative agents.

Many microorganisms have been described. Marcus Rabinovitch[1] described a Gram positive diplobacillus which he cultivated from cases in an epidemic in Kieff and with antigens prepared from this organism he obtained complement-fixation and agglutination. Fürth studied an epidemic in China and obtained short, plump rods which grew aerobically in short chains, which were first looked upon as diplococci. P. Th. Müller saw a diplobacillus upon which he did not lay much stress etiologically, and von Prowezek described inclusions in leucocytes which he regarded as protozoa. It is hardly worth while at the present time to describe in detail the many different findings that have been reported, since in few of them is there sufficient evidence to enable us to come to conclusions.

In 1914 Plotz[2] described a short Gram positive bacillus which he obtained

by anaerobic cultivation, with much regularity, from cases of Brill's disease at the Mt. Sinai Hospital, New York, and which has been made the subject of considerable study by Plotz, Olitsky and Baehr.[3] They have obtained the bacillus again and again, have succeeded in obtaining positive agglutination and complement-fixation in the blood of endemic typhus cases after the crisis, and have obtained a similar bacillus from a number of European typhus cases which have come into quarantine.

The method of cultivation by which this bacillus is obtained is relatively simple, consisting of taking blood directly from a vein into high tubes containing glucose agar and unheated and unfiltered ascitic fluid of a specific gravity not less than 10, 15. The American Red Cross Commission which went to Serbia during the last typhus epidemic—and of which the writer was a member—attempted to work along the lines laid down by Plotz but found it extremely difficult to do systematic work and obtain reliable materials under the conditions then existing. The undersigned obtained an organism very similar to the Plotz bacillus by Plotz's method in two cases. In the first of these the organism could not be carried further than the second generation, and in the second it did not reach America alive. Hopkins obtained a similar organism later toward the end of the epidemic, which is still under cultivation. However these organisms were found so rarely in spite of a considerable number of blood cultures that were taken, and the necessity of working with unsterilized ascitic fluid under relatively primitive conditions compelled the judgment that these isolated findings, though pointing somewhat in favor of Plotz's organism, could not be taken as warranting conclusions. However, of the many blood cultures taken, the smaller number were taken from fresh cases, most of the ascitic fluid used did not come up to the specifications of Plotz, and there is no doubt about the fact that the organism described by Plotz is not one that is easily cultivated unless rich ascitic fluid is available.

The fact that the Plotz organism has been cultivated from guinea-pigs and monkeys experimentally inoculated by the workers at Mt. Sinai, as well as by Anderson, is further evidence in favor of this bacillus.

Petruschky[4] has recently cultivated a similar but aerobic bacillus from sputum in typhus cases and Arnheim[5] has cultivated an organism which in appearance and staining properties is not unlike the Plotz bacillus, but also grows aerobically. Arnheim obtained his organism from six cases, on ascitic agar plates, on which on the first cultures there appeared a growth hardly visible to the naked eye, which in transplants continued to grow very delicately. He states that his organism is not unlike that of Petruschky and that he obtained it out of the blood, the sputum and the urine of typhus cases.

Judgment on all these many different findings is complicated by the fact that the undersigned, as well as others, has found that typhus cases in the later stages of the disease are often secondarily infected and streptococci and other organisms can occasionally be cultivated from the blood in the later stages and from the spleen at autopsy. Moreover the fact that the organisms most persistently described are in appearance and staining properties so similar to the so-called *diphtheroids,* which are so often obtained, with doubtful etiological significance, from lymphoid tissues, blood and ascitic fluid intro-

duces an element of doubt which should lead to the greatest conservatism. This applies, of course, more strongly to the organism isolated by Plotz, in which it is apparently indispensable to use unheated and unfiltered ascitic fluid. However, in the work of Plotz, Olitski and Baehr, this element seems to have been very carefully controlled by many blood cultures taken in nontyphus cases with the same ascitic fluid used in the blood cultures taken from patients.

Of all the organisms so far under consideration, that of Plotz seems to be the most important, but final judgment must await results obtained in the work now being carried on by Plotz and others thoroughly familiar with the method, in Europe and in other places.

The fact that the organism within one generation seems to lose entirely, or almost so, its virulence and that so far vaccination of animals has not protected them against inoculations with the virus is a point which justifies reasonable doubt. On the other hand, failure to vaccinate, or even to actively immunize, is not conclusive proof against the etiological significance of an organism. It seems established with fair definiteness that a bacillus like that of Plotz can be cultivated from many cases of typhus exanthematicus and there seems to be a strong possibility that the organism causes the disease. The work that has been done on it has been done with great care and a desire to get at the truth, and the work at present being continued in this spirit, with available typhus material in Europe, probably by a number of independent workers familiar with the disease and under conditions of well equipped laboratories, should bring conclusions which before long will permanently settle the question of the etiological significance of the Plotz bacillus.

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—*H. Z.*

Tumor Problems

THE fact that very many laboratory workers in all parts of the world are studying tumors, and that the experimental results are published in very many journals, many of which are inaccessible to men who are not spending most of their time in laboratories where the literature is accessible, makes a general discussion of the problem of cancer research of considerable value. Such a discussion is furnished by Ewing.[1]

Ewing comments at some length upon the problems presented during researches upon the transmissibility of animal tumors. A prominent problem, he says, is whether all of the tissues used in such experiments are true neoplasms, and in connection with this he quotes Virchow's remark, which many investigators and students forget, to the effect that no one, even under torture, can state exactly what a tumor is. The older notion, that a true neoplasm is only viable in its host, we now know is not true, and this fact makes it increasingly difficult to separate the infectious growths from the noninfectious. The morpho-

logic criteria (and these are practically the only ones we have as yet) of malignancy are the following:

First, and most important, the atypical qualities of tumor cells. In the absence of other characters this alone, when sufficiently developed, determines the fact of malignancy. The degree of variation from the normal type of cell from which the growth originates is what one means in this instance.[2]

Second,—the loss of polarity. In normal tissues there is a regular relationship of the cells to one another, or to basement membranes, which is lost as cells become atypical and take on malignant characters.

Third,—infiltration, by which is meant the power of invading and in part destroying the invaded tissue.

Fourth,—metastasis, which is usually pathognomonic of malignancy, but which may occur in almost any type of benign growth.

Fifth,—the capacity to excite the growth of connective tissue. This property denominated desmoplastic, is not constant.

Sixth,—hyperplasia, a symptom which is difficult to delineate because of the grades of hyperplasia which result from irritation and functional causes.

Judged by these criteria, says Ewing, the tumors of the lower animals are true neoplasms. Speaking of the Rous chicken sarcoma, he seems to have no doubt. The fact that this growth can be transmitted by using filtrates from porcelain filters, is interesting but not conclusive that the process is infectious. Experimental criteria for neoplasms include bacterial sterility and inability to be propagated by filtrates. Rous' tumors are sterile. Ewing believes that in the case of these tumors one is dealing with a chemical transmissible virus. This point of view in its application to tumors is an exceedingly interesting one for which undoubtedly much might be said. It will be sufficient, however, to merely mention the fact that there is in the work on "Vitamines" evidence that exceedingly small amounts of matter may be able to produce exceedingly large effects, and that it is entirely possible that in the case of tumors there is a substance (or substances) which even in infinitessimal amounts may incite cells to growth and continuous overgrowth. It is possible that it is the physical condition of such substances which accounts for their activity rather than the mere chemical structure.

Ewing calls attention to another point of view that deserves accentuation. This refers to the possibility of variation of standards for animal and human tumors, examples of which possibility he cites. He refers briefly to other aspects of the cancer question, such, for instance, as the effects of constituents of dietaries,—carbohydrates, lime; and to the effects of x-rays and radium. In speaking of the influence of age, he refers to the fact that whereas cancer has been considered a disease of old age, nevertheless young animals have been found to furnish a better soil for the growths in experimental work. He refers to the problem of associated lymphocyte change and the growth of tumors.

This review of cancer problems, Ewing says, inclines him to believe that each neoplasm should be considered as a specific disease, each type differing in its etiology, clinical course and therapeutic possibilities. He compares the neoplasia as a group with the infectious diseases as a group, and points to the fact that in the latter the general features may be alike yet the causes are different.

We might note especially the similarities in the group of granulomas. It may indeed be possible that though the carcinomas appear alike, they are etiologically dissimilar. Perhaps it is for this reason that excision is the only satisfactory method we have yet of dealing with tumors, and that other therapeutic measures have failed in universal useful application.

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—*P. G. W.*

Recent Developments in the Therapeutics of Lobar Pneumonia

IN the study of few of the common bacterial infections has there been more cooperative activity of recent years than in that of lobar pneumonia. The intensive study of pneumococcus immunity by Neufeld, a study covering many years and optimistically continued in spite of many discouragements, finally yielded important results in 1910. Neufeld and Haendel were able to show that an important element in the failure of previous workers to influence pneumococcus infections by serum therapy had depended upon the fact that the pneumococcus group included more than one organism, and that the different types, though morphologically and culturally entirely alike, were serologically strictly distinct. The antibodies produced in an animal by immunization with one strain had practically no activity upon cultures of another strain. In consequence, no serum therapy could be expected to exert any influence unless a serum was used which had been produced with a strain of organisms identical in type with that infecting the patient.

This line of investigation has been followed extensively at the Rockefeller Hospital, where in the laboratory of Cole, Dochez and Gillespie succeeded in making a preliminary classification of pneumococci into four types. Type I and Type II are apparently well-defined groups, the members of which react specifically with sera of their respective types. Type III is the pneumococcus mucosus and Type IV is a temporary "attic" into which heterogeneous types not reacting with sera I or II and not interrelated among themselves are conveniently stored for the present. Types I, II, and III have been found to be associated most frequently with disease, whereas Type IV, though also very common in pneumonia, is nevertheless less common than the preceding and apparently less serious prognostically. A serum powerful enough to influence infections with Type I has been successfully produced, and this serum, injected intravenously, while apparently not influencing the process in the lung, nevertheless will sterilize the blood and therefore exert a powerfully beneficial action in severe cases. Although the serum treatment of Type I pneumonia is still more or less in the experimental stage, it is being extensively used at the Rockefeller Hospital and in other hospitals which have access to the serum there produced. And since in the opinion of Cole and others who have employed it, the use of the serum represents a probably very important advance in therapy, it becomes very important for hospital laboratories and practitioners of medicine to be familiar with the method at present in use for the determination of the pneumococcus type.

The method at present in use and based on the work carried out at the Rockefeller Hospital is as follows:

The patient is required to wash out his mouth with some antiseptic, and then expectorate sputum, obtained by coughing, into a Petri dish. The sputum is washed by lifting it through a series of watch glasses containing sterile salt solution, is then ground in a mortar with a little salt solution, and the even emulsion so obtained is injected intraperitoneally into a white mouse. A fine needle is carefully introduced upward and inward from just above Poupart's ligament. Puncture of the liver may be avoided in this way. The pneumococci develop in the peritoneum, and after four to eight hours the mouse is killed and the peritoneal cavity carefully washed with salt solution by means of a capillary pipette. The washings are placed in a centrifuge tube and certifugalized at low speed for several minutes to bring down leucocytes. The supernatant fluid is then centrifugalized at high speed and the pneumococci so obtained are resuspended in salt solution. This suspension is added to equal parts of the sera of Types I and II in dilutions of 1 to 2½ and 1 to 10, with, of course, proper controls and a bile test to determine bile solubility. Agglutinations can also be made later with broth cultures made from the mouse's heart's blood or peritoneal exudate.

The agglutinations can be confirmed by protection experiments in mice, but this is usually unnecessary in hospital work, since the agglutinations sharply determine whether the organisms belong to Type I or II, or, by elimination, to Type IV. If of Type I, serum treatment can be instituted. In the case of Type II, it is not improbable that such treatment may be possible in the near future. If the organism belongs to Type IV, a certain amount of prognostic information is gained.

—*H. Z.*

Diuresis

THE mechanism of diuresis is unsolved, probably because the mechanism of urinary secretion is unsolved. It may perhaps be possible that light upon this subject may be shed by studying diuresis and applying the discovered facts to urinary secretion.

It has been assumed that during the secretion of urine a protein-free aqueous solution of salts is formed in the glomeruli and that this solution in its passage through the renal tubules becomes concentrated by reabsorption of water, and by the addition of urea, uric acid and certain salts.[1] It has been assumed, on the one hand, that the fluid in the glomeruli is a pure filtrate, or, on the other, that it is formed as an expression of secondary activity of the glomerular epithelium. The one assumption necessitates the action of pressure; the other postulates a factor or factors of which nothing is known, unless we engraft upon it a physicochemical conception which takes account of absorption. Against the filtration theory stands the fact that there never is sufficient pressure difference to permit of rapid enough transmission of water from a colloidal sys-

tem like the blood, through a colloidal membrane such as the glomerular capillary walls.

The more recent conception of Fischer[2] seems for the present at least, to take account of all the conditions in a more satisfactory manner than the older theories. According to Fischer, urinary secretion depends essentially upon the presence of free water in the blood of the kidney; that only when the water is free can it pass the colloidal walls of the renal capillaries or tubules under the conditions existing in the kidney. If water in colloidal combination be added to the blood, as, for instance, in the form of blood serum, urinary secretion is not affected to any appreciable extent no matter how much serum is introduced. This is the basis of Hogan's treatment of shock.[3] But even if there is free water in the blood, little of it will pass into the glomeruli unless the chemical conditions in the various colloids in the kidney are properly balanced. If there is too much acid in the blood, the water will be held more closely to that fluid; if the acid content of the blood falls; i. e., if the oxygen content is raised, the water will become free for excretion. In other words, it is only when the tissue of the kidney which borders on the blood has a relatively higher acid content than the blood itself that water can be taken from the blood and started in its excretory path. If the blood flow through the kidney is slow and the oxygen content is low, secretion also will be low; if it is rapid, and the blood is well aerated in the lungs, the oxygen content of the arterial blood in the kidney will increase, the acid content will be lower, and secretion will increase. The diuretic drugs, such as caffeine, act by increasing the arterial flow through the kidney. The diuretic salts act by counteracting the effects of acids in the tissues.[4]

If the colloidal theory of secretion is true, then all substances which produce diuresis must do either or both of two things; they must increase the arterial blood supply through the kidney, or they must act in a physicochemical way upon the renal colloids and cause them to give up water.

Cow[5] has recently observed that physiologic amounts of saline solutions, injected into the blood stream, produce no increase in diuresis, while if the same amounts are given by mouth a diuresis occurs. This led him to suggest that the water taken by mouth accumulated some substance from the mucosa of the gastrointestinal tract, which had a diuretic effect, and that that substance, directly or indirectly, is largely concerned in the production of diuresis. He observed that extracts of duodenal mucous membrane, when used for perfusing the kidney, caused a diuresis, provided the perfusion fluid previously had been pressed through the vessels of the head and neck. Passage through a headless animal was ineffective. These observations suggested that the pituitary secretion played a part in the problem. The process, as Cow conceives it, would be something like this,—water enters the gastrointestinal tract and during absorption takes something from the mucosa of the duodenum. This in turn is carried to the pituitary which is stimulated to produce its substance which has an effect upon the urinary secretion. This is an interesting contribution which, as has been suggested[6] recalls the mechanism of pancreatic secretion. But after all it merely states the possibility that extracts of duodenal mucous

membrane may have an effect in causing the pituitary to secrete. Emotions of one or another type or grade may do the same thing. It helps the problem of renal secretion not at all. It, however, seems to emphasize the fact that renal secretion is a chemical problem and that it does not depend upon the nerves. In partial support of this stands the work of Pearce and Carter[7] who have shown that so far as the vagus is concerned, the oxygen consumption of the kidney is not changed by stimulation of that nerve. This work also shows that renal activity is primarily dependent upon the use of oxygen.

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—*P. G. W.*

Chloroform Necrosis of the Liver.

IT has been known for some time that chloroform necrosis may be followed by pathologic changes in the liver, and by a series of symptoms which simulate acute yellow atrophy. It is known from the writings of Wells,[1] Whipple,[2] Graham[3] and others, that these changes depend upon the fact that chloroform reduces the oxidation processes of the body. In connection with the work upon this subject it was reported first by Whipple[2] that whereas adult experimental animals were susceptible to chloroform, and became the subjects of chloroform liver necrosis, young animals, i.e., ones within three weeks of age, were not susceptible. Graham[4] has attempted to discover why there is this difference between adult and young animals. He has been able to corroborate Whipple's observation of the relative insusceptibility of pups, and feels that he has evidence that this insusceptibility is due to the glycogen content of the livers of the animals. This evidence depends upon the fact that starvation or phlorhizination which depletes the glycogen, produces pups which show liver necrosis after chloroform; that increasing the glycogen content of the liver of adult animals renders them less susceptible to chloroform necrosis; and that in well-nourished pups the glycogen content of the liver may be as high as 9.07 per cent.

In another article Graham[5] shows that chloroform necrosis depends upon the formation of hydrochloric acid from chloroform, but he is not able to account for the inhibitory effects of glycogen. And yet the experiments which he gives are convincing and lead to the very important therapeutic suggestion that the feeding of carbohydrates to adults lessens their susceptibility to liver necrosis by chloroform.

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—*P. G. W.*